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<b>(21) International Application Number:</b> PCT/EP00/00598 <b>(22) International Filing Date:</b> 26 January 2000 (26.01.2000) <b>(30) Priority Data:</b> 99101480.4 27 January 1999 (27.01.1999) EP <b>(60) Parent Application or Grant</b> IDEA AG [/]; (). CEVC, Gregor [/]; (). CHOPRA, Amla [/]; (). STIEBER, Juliane [/]; (). CEVC, Gregor [/]; (). CHOPRA, Amla [/]; (). STIEBER, Juliane [/]; () . VOSSIUS & PARTNER; ().		<b>Published</b>
<b>(54) Title: TRANSNASAL TRANSPORT/IMMUNISATION WITH HIGHLY ADAPTABLE CARRIERS</b> <b>(54) Titre: TRANSPORT OU IMMUNISATION TRANSNASALE A PORTEURS HAUTEMENT ADAPTABLES</b>  <b>(57) Abstract</b> <p>The invention deals with the transport of preferably large molecules across nasal mucosa by means of specially designed, highly adaptable carriers loaded with said molecules. One of the purposes of making such formulations is to achieve non-invasive systemic delivery of therapeutic polypeptides, proteins and other macromolecules; the other intent is to overcome circumstantially the blood-brain barrier by exploiting the nasal cavity to enter the body and then to get access to the brain. A third intent is to achieve successful protective or tolerogenic immunisation via nasal antigen or allergen administration.</p> <b>(57) Abrégé</b> <p>L'invention concerne le transport de molécules de préférence grandes à travers la muqueuse nasale au moyen de porteurs hautement adaptables, conçus spécifiquement et chargés de ces molécules. L'un des objets de telles préparations est d'obtenir une diffusion systémique non invasive de polypeptides, protéines et autres macromolécules thérapeutiques. L'invention vise également à surmonter accessoirement la barrière hémato-encéphalique en exploitant la cavité nasale pour entrer dans le corps, puis accéder au cerveau. L'invention vise en outre à atteindre une immunisation tolérogénique ou protectrice réussie par administration nasale d'antigène ou d'allergène.</p>		

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<b>(54) Title:</b> TRANSNASAL TRANSPORT/IMMUNISATION WITH HIGHLY ADAPTABLE CARRIERS  <b>(57) Abstract</b>  The invention deals with the transport of preferably large molecules across nasal mucosa by means of specially designed, highly adaptable carriers loaded with said molecules. One of the purposes of making such formulations is to achieve non-invasive systemic delivery of therapeutic polypeptides, proteins and other macromolecules; the other intent is to overcome circumstantially the blood-brain barrier by exploiting the nasal cavity to enter the body and then to get access to the brain. A third intent is to achieve successful protective or tolerogenic immunisation via nasal antigen or allergen administration.		

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Description

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### Transnasal transport/immunisation with highly adaptable carriers

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The invention deals with the transport of preferably large molecules across nasal mucosa by means of specially designed, highly adaptable carriers loaded with said molecules. One of the purposes of making such formulations is to achieve non-invasive systemic delivery of therapeutic polypeptides, proteins and other macromolecules; the other intent is to overcome circumstantially the blood-brain barrier by exploiting the nasal cavity to enter the body and then to get access to the brain. A third intent is to achieve successful protective or tolerogenic immunisation via nasal antigen or allergen administration.

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Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention. Further incorporated by reference is the complete disclosure content of the co-pending application filed in the name of IDEA AG and bearing the title "Noninvasive vaccination through skin".

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Nasal delivery has been explored extensively over the last decades and was discussed repeatedly as an alternative to the systemic delivery of drugs, especially peptides and proteins, which normally must be injected. Nasal delivery also attracted interest owing to the fact that it avoids the hepatic first-pass effect, the problem of degradation in nasal cavity notwithstanding, which creates a pseudo-first-pass effect (Sarkar, 1992). The latter difficulty prompted chemical or recombinant structural peptide or protein modifications to improve the stability and minimise the enzymatic cleavage of macromolecules in the nose (Wearley, 1991).

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Some earlier reviewers (Illum, 1991; Wearley, 1991) expected that transnasal peptide delivery, supported by absorption enhancers, will provide a convenient, efficient means for the administration of protein and peptide therapeutics. More recent surveyors took less optimistic stance, however (Harris, 1993). Rapid metabolism and nonlinear pharmacokinetics of nasally delivered peptides (Wearley, 1991) are partly responsible for this. The other reasons are the anatomical and temporal barriers presented by the

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nasal mucosa (Sarkar, 1992), and especially the intolerable side effects of most, if not all, methods currently in use for nasal delivery. This holds also true for efforts to deliver compounds with the aim to generate a protective immune response transnasally, which would represent a more natural way of antigen presentation than encountered by conventional injection. The adverse side effects observed with transnasal immunisation experiments are mainly due to the presence of immunoadjuvants (such as Cholera toxin (CT) or its fragment B, heat labile protein from *E. coli*, keyhole limpet hemocyanin, or other substances with ADP-ribosylating activity, for example), and/or molecules with a permeation enhancing activity, in addition to the antigen in the formulation for nasal delivery. While the former may be toxic, the latter are irritating to the immunised subject. Selectivity of immune response, moreover, cannot be achieved with unspecific stimulatory agents. Moreover, there is substantial variability in the resulting immune response after nasal antigen administration, probably due to the difficulty of depositing the immunogen on the sites in the nasal cavity with the lowest transbarrier transport resistance.

The human nasal cavities with a total volume of 15 mL and a total surface area of 150 cm<sup>2</sup> - which amounts to more than 1 m<sup>2</sup> if one allows for the surface corrugations - are covered by mucus and a mucosa 2 mm to 4 mm thick. Most of the cavity surface is lined by a respiratory epithelium, comprised of columnar cells, goblet cells, and ciliary cuboidal cells. The resulting permeability barrier is related to that of the oral cavity, with which it communicates and which is covered by a keratinised barrier tissue. In either case, the cells in the barrier are tightly packed and often sealed with the specialised intercellular lipid arrangements. Moreover, in either case, the permeability barrier is lowered by the topical use of substances which compromise the quality and packing of such lipidic seals and/or which increase the probability for molecular partitioning into the barrier. Deviant from the situation encountered in the mouth, from the nose foreign substances are cleared into the nasopharynx by the cilia, with an average speed of 5 mm/min. An exception is the upper region of nasal cavity, which contains no cilia but is covered by a pseudo-stratified olfactory neuroepithelium. The nasal subepithelium contains a dense vascular network and the venous blood from the nose passes directly into the systemic circulation.

Nasal route of delivery has been relatively unsuccessful to date when used for high molecular weight substances. Use of permeation enhancers did not improve the

5 situation sufficiently, largely due to the fact that such substances are generally poorly  
tolerated and of limited usefulness. The pharmacodynamics resulting from nasal drug  
delivery is also highly variable. Major reasons for this are the inconsistency in the site  
10 of deposition or in the delivery details, as well as changes in the mucous secretion and  
mucociliary clearance; the latter are compounded especially by the presence of allergy,  
hay fever, and the common cold in treated subjects (Harris, 1993). Protein degradation  
in mucosa is important as well (Sarkar, 1992). Despite this, numerous studies were  
15 done with buserelin, vasopressin, cholecystokinin, calcitonin, growth hormone and  
related substances (e.g. GHRH), erythropoietin, G-CSF, interferon, insulin,  
gonadotropin hormone releasing hormones (GnRH), and vasopressin analogues, the  
results of which are reviewed briefly in the following.

#### 20 **Systemic delivery of large drugs through the nose**

25 Hexarelin (GH analogue; MW = 800). The GH response to the intranasal hexarelin  
administration (about 18  $\mu\text{g/kg}$ ) was not significantly higher than that induced by an  
injection of 1  $\mu\text{g}$  GHRH/kg (Ghigo et al., 1996). On the other hand, the former kind of  
treatment did not significantly modify IGF-I but increased IGFBP-3 levels. Both IGF-I  
30 and IGFBP-3 levels were slightly but significantly increased by oral treatment with the  
drug as well (Ghigo et al., 1996).

35 Intranasal treatment with octreotide nasal powder, a somatostatin analogue (up to 2  
mg TID, corresponding to a mean GH value below 5  $\mu\text{g/L}$  during 8 daytime hours), was  
well tolerated, with only mild side effects and no significant changes in the nasal  
mucosa. An improvement of the clinical picture was registered in all patients after a  
40 few days of octreotide nasal powder administration. Positive correlation was found  
between GH and IGF-I, GH and IGFBP-3, IGF-I and IGFBP-3, insulin and IGFBP-3  
and insulin and IGF-I during chronic (3-6 months) treatment (Invitti et al., 1996).

45 Cholecystokinin (MW = 1050). The carboxy terminal octapeptide of cholecystokinin  
(CCK-8) has similar functions as native cholecystokinin (CCK), but lacks receptor  
selectivity and metabolic stability. Mediation of satiety via the A-receptor subtype can  
50 be used for management of obesity. This was also shown after intranasal  
administration of Hpa(SO<sub>3</sub>H)-Nle-Gly-Trp-Nle-MeAsp-Phe-NH<sub>2</sub>, the result of moving

the N-methyl group from Phe to Asp, which inhibited feeding in beagle dogs (Pierson et al., 1997).

After intranasal (10  $\mu$ g) and intravenous (0.25  $\mu$ g and 2.5  $\mu$ g) administration of an octapeptide derivative of cholecystokinin, the substance CCK-8 was shown to affect the auditory event related potential (AERP) in 20 healthy subjects. The effect was stronger in women than in men (Pietrowsky et al., 1996). Plasma CCK-8 concentrations after intranasal administration of 10  $\mu$ g CCK-8 were comparable to those of 0.25  $\mu$ g CCK-8 given i.v., but were substantially lower than those elicited by 2.5  $\mu$ g CCK-8 (Pietrowsky et al., 1996).

Vasopressin (MW = 1054). Vasopressin DGAVP (2 mg) was administered intranasally and orally to healthy subjects for 1 week. Peak levels were always observed at 15 min. The mean absorption and elimination half-life (around 8 min and 35-38 min, respectively) were similar for the two tested routes of administration, but the latter only had 0.7 % relative bioavailability (Westenberg et al., 1994).

In a double-blind, crossover study, subjects received on three different occasions 20 IU of (arginine)vasopressin (AVP) intranasally (IN), or 1.5 IU of AVP and saline solution i.v. Evoked potentials (ERPs) were recorded during the subject's performance on a auditory attention task. Plasma concentrations of vasopressin during task performance were enhanced after AVP, with the increase after i.v. administration of AVP exceeding 2000-fold that after AVP i.v. Intranasal administration of AVP substantially increased the P3 component of the ERP in contrast to the injection (Pietrowsky et al., 1996).

Acute (2 mg) and chronic, 2 weeks treatment (1 mg/day) with nasal DGAVP revealed an improved short-term memory for abstract words in males but not in females, with no positive effect on learning concrete words. Chronic, but not acute, treatment with DGAVP reduced the reaction time for scanning of digits in a memory comparison task (Sternberg paradigm) in both sexes (Bruins et al., 1995). In a different human study, arginine-vasopressin (AVP: 3x10 IU) enhanced memory performance after nasal administration. The late positive complex (LPC) elicited by oddball stimuli was not affected whereas the structural encoding task revealed an effect of the drug. In both studies, AVP intake resulted in a marked change of the scalp distribution of the P3 component, which is a prominent part of the LPC. Vasopressin was thus concluded to



influence the central nervous processing of the emotional content of stimuli (Naumann et al., 1991).

Subchronic treatment with vasopressin (40 IU/day) was shown to enhance nocturnal slow-wave sleep in 2 elderly subjects (Perras et al., 1996). However, the intranasal administration of vasopressin (DDAVP: 30 or 60 micrograms) had no general effect on pain perception in humans, but some other effects were observed (Pohl et al., 1996).

Buserelin (MW = 1239). Treatment of 40 women with endometriosis and 10 women with uterine leiomyoma by using GnRH agonist buserelin (200 µg, 3x daily, 6 months, intranasally) reduced AFS mean pelvic score from 24 to 7 and the size of the fibroids decreased by 69 % (Biberoglu et al., 1991).

Calcitonin (MW = 3432). Ichikawa et al. (1994) concluded that nasal (5, 10, 20 and 40 U/rat) and subcutaneous (5, 10 and 20 U/kg) administration of *Salmon calcitonin* on alternate days for 3 weeks, starting a week after ovariectomy, prevented the osteopenic changes, the invasive method being approximately 2-times more effective.

In a double-blind trial, the effect of intranasal administration of *Salmon calcitonin* on biochemical parameters of bone turnover in 32 patients immobilised for a prolapsed intervertebral disk was investigated (van der Wiel et al., 1993). Calcitonin in a dose of two times 200 IU/day inhibited by 40 % the increase in the fasting 2 h urinary hydroxyproline/creatinine ratio (OHP/Cr) and lowered by 80 % the increase in calcium/creatinine ratio (Ca/Cr). The decrease in serum 1,25-dihydroxyvitamin D after 10 days of immobilization was significantly less in the calcitonin-treated group than in the placebo group (14 versus 29%, respectively;  $P < 0.05$ ). However, intranasal calcitonin, which was well tolerated, did not influence the pain scores as measured with a visual analog scale (van der Wiel et al., 1993).

Growth hormone (GH) releasing factor/s (MW = 5040). The current mode of growth hormone replacement therapy is daily subcutaneous (s.c.) injections given in the evening. This schedule is unable to mimic the endogenous pulsatile pattern of GH secretion, which might be of importance for the induction of growth and other GH actions (Laursen et al., 1996).

5 To simulate endogenous production of growth hormone the protein was administered  
on three occasions intranasally in doses of 0.05, 0.10 and 0.20 IU/kg, using  
didecanoyl-L- $\alpha$  -phosphatidylcholine as an enhancer (Laursen et al., 1996). On the  
10 other two occasions the patients received an s.c. injection (0.10 IU/kg) and an i.v.  
injection (0.015 IU/kg) of GH, respectively. The nasal doses and the s.c. injection were  
given in random order in a crossover design. Intravenous administration produced a  
short-lived serum GH peak value of 128  $\mu$ g/L. Peak levels were around 14  $\mu$ g/L after  
15 s.c. injection (50% bioavailability) and between 3  $\mu$ g/L and 8  $\mu$ g/L, respectively, after  
the three nasal doses (bioavailability between 4% and 9%). Serum insulin-like growth  
factor I (IGF- I) levels increased significantly after s.c. administration only. However,  
the data revealed that a closer imitation of the physiological GH pulses was achieved  
20 via the nose. Despite this the authors of the study concluded that GH administration is  
of limited importance for the induction of a metabolic response to GH (Laursen et al.,  
1996).

25 GHRP-2 is one of the most potent members of the GHRP family, which exerts its  
biological activity after oral, intranasal and i.v. administration. For example, the children  
who had a robust response to the injected GH-releasing factors also received  
30 intranasal GHRP-2, with significant, but not quantitated, response over a dose range of  
5-20  $\mu$ g/kg per dose (Pihoker et al., 1995).

35 Insulin (MW = 5808). The problem of low bioavailability of insulin solutions given  
through the nasal mucosa was improved by using absorption enhancers or  
bioadhesive microspheres (Gizurason & Bechgaard, 1991; Illum & Davis, 1992).  
Bioavailability greater than 10% was measured but to date no corresponding  
40 formulation has found its way into the late clinical trials. The chief reason for this  
appears to be the severe damage to nasal mucosa caused by the commonly used  
permeation enhancers.

45 For example, following the administration of powder formulations comprising insulin  
and the permeation enhancer sodium tauro-24,25-dihydrofusidate (STDHF), the  
hypoglycaemic response and the serum insulin levels in sheep increased with  
STDHF/insulin molar ratio in the range 0 to 16.8 (Lee et al., 1991). The reason for this  
50 is increased mucosal permeability as well as reduced insulin aggregate size. The  
bioavailability ranges from 2.9% to 37.8% for the powder, and was reported to be

15.7% and 37.4%, respectively for the drops or spray containing STDHF/insulin = 8.4/1 mixture, and roughly proportionally to the enhancer concentration (Lee et al., 1991). To achieve a high bioavailability major changes in nasal mucosa had to be tolerated, however.

In humans, the 200 U insulin/mL formulation containing a blend of enhancers (didecanoyl-phosphatidylcholine (2 w-%), glycerol (1.6 w-%), 0.4 w-% fractionated coconut oil) and 0.2 w-% cholesterol resulted in appr. 8 % bioavailability, the highest values having been measured for the high dose (2x3 sprays of 50  $\mu$ L each), which also was most irritant (Drejer et al., 1991).

Cyclodextrins dissociate insulin hexamers into smaller aggregates, in dependence on structure and concentration. Hexamer dissociation was therefore speculated to be the reason for higher nasal absorption of the polypeptide (Shao et al., 1992). The relative effectiveness of various cyclodextrins for this purpose was reported to decrease from dimethyl- $\beta$ -cyclodextrin (DM- $\beta$ -CD) >  $\alpha$ -cyclodextrin ( $\alpha$ -CD) >  $\beta$ -cyclodextrin ( $\beta$ -CD), hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) >  $\gamma$ -cyclodextrin (gamma-CD). A direct relationship between absorption promotion and nasal membrane protein and lipid release was invoked to explain such sequence (Shao et al., 1992).

It is less clear why cationic chitosan enhances the absorption of insulin across the nasal mucosa of rat and sheep in a concentration dependent fashion, with optimum concentrations higher than 0.2% and 0.5% in rats and sheep, respectively, but overall efficiency of this procedure is only around 10% (Illum et al., 1994). Using didecanoyl-L- $\alpha$ -phosphatidylcholine as an enhancer results in 4% to 9% of nasal insulin bioavailability (Laursen et al., 1996).

G-CSF (MW = 19600). The relative bioavailability of rhG-CSF administered nasally in the rat was approximately 2%, compared to an s.c. injection, as evaluated from the immunologically active rhG-CSF concentration in rat plasma and the area under the curve (AUC) at t=8 h. Leukocyte stimulation counts suggested 5-10% availability at t=48 h. Relative bioavailability and pharmacological availability were increased 23 times and 3 times, respectively, by polyoxyethylene 9-lauryl ether (Laureth-9), but no increase in availability occurred with sodium glycocholate (Machida et al., 1993).

5 Absorption of dissolved recombinant human granulocyte colony-stimulating factors (rhG-CSF at pH 4) through the nose of rabbits was investigated with dimethyl- $\beta$ -cyclodextrin added or without such excipient, which acts as barrier permeation  
10 enhancer. The proteins were absorbed and the total leukocyte numbers in peripheral blood increased in either case, but excipients improved the absorption of rhG-CSF appreciably (Watanabe et al., 1993). A subsequent pharmacokinetic and  
15 pharmacodynamic study (Watanabe et al., 1995) revealed that protein is absorbed through the nasal cavity from a solution, especially in the presence of  $\alpha$ -cyclodextrin ( $\alpha$ -CyD), which can act as carrier in the membrane. Good correlation was  
20 found between the logarithm of the area under the serum G-CSF concentration-time curve (AUC) and the area under the increased total blood leukocyte count-time curve (Watanabe et al., 1995).

25 Interferon (MW = 23000). Treatment of experimental rhinovirus colds in 38 adults by intranasal administration of recombinant interferon beta serine (MW = 18500) had no effect on illness rate or severity, but did decrease the frequency of virus shedding by  
30 the factor of 2 (on day 4) to 3 (on day 6). The course of middle-ear dysfunction associated with experimental colds was also positively affected by the drug (Sperber et al., 1992).

35 Erythropoietin (MW = 30400). The pharmacological availability of rh-EPO after intranasal administration without enhancers was compared to that of intravenous injections. The pharmacological activity was enhanced in low pH and hypotonic  
40 mannitol solution, which both compromise the barrier quality. This resulted in relative bioavailability of nasally applied drug between 7% and 4%, when estimated by different reticulocyte counting methods. (Shimoda et al., 1995).

45 Labelled dextrane (MW = 4100, 9000, 17500), applied nasally at the dose of 6.5 mg, was seen to pass mucosa in the presence of glycocholate (3 mg) and found in the blood in concentration range between 6 ng/mL and 21 ng/mL, which corresponds to  
50 app. 0.05%, 0.02%, and 0.01% for the three molecular sizes, respectively (Maitani et al., 1989).

5 In summary, the combined teachings of the prior art demonstrated that the likelihood of large molecules to pass nasal mucosa decreases strongly with increasing molecular weight. To date, the size of molecules administered successfully through the nose is typically < 1300 Da, and always below 3500 Da. Significant transport is achieved only  
10 with supporting permeation promoters and is, in a certain concentration range at least, proportional to the enhancer concentration. Enhancer concentration in the percentile range can ensure up to 30% drug (or label) bioavailability but more often values below 10% and typically of a few percent are obtained. High transfer efficiency is  
15 accompanied with strong local tissue damage. This causes unpleasant acute side effects and may, first, abrogate the nasal permeability barrier and, upon repeated use, provoke extensive keratinisation of the epithelium that finally reduces transnasal transport efficiency.  
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25 The success of transnasal transport is believed to rely on the loosening of ciliated-goblet, goblet-goblet, or ciliated-ciliated cell contacts, which also opens passages for the motion of water (McMartin et al., 1987). Procedures or substances which support the process either osmotically (as in the case of polysaccharide addition), physico/chemically (as in the case of surfactant addition) or biologically (as in the case  
30 of molecules which affect the cell biochemistry, including many drugs, cell adhesion or trans- and epicellular transport), can therefore improve drug delivery across the nasal mucosa. Translocation through the cells is possible, but probably rare, except, maybe, in the cases of some viral infections or applications. Materials, such as polymers of polyelectrolytes, which prolong the retention time of and increase the proximity  
35 between the molecules to be transported and cellular membranes, are useful for the purpose as well. The limit to this latter effect is set by ciliary motion, which tends to clear mucosal surface approximately every 30 min and transports the superficial material into the throat, and thus towards the gastrointestinal tract. Transport mediated  
40 by certain particles was contended to rely on this effect.

#### 45 **Particle delivery through the nose**

Inhaled fine particles (Kanto loam dust, fly ash, carbon black, diesel exhaust particles (DEP), and aluminium hydroxide (alum)) appear to act as adjuvants, and accelerate  
50 the production of IgE antibody against pollen in female BDF1 mice; however, the

5 nature of the particles, their capacity to adsorb antigens, and/or their size seem to play only minor role in the process (Maejima et al., 1997).

10 Hollow spheres, according to Ting et al. (1992), are unsuitable for nasal delivery, owing to their rapid clearance and variable deposition pattern. Polyvinyl alcohol microparticles in the form of collapsed, solid spheres with the desired size for nasal deposition (10-200  $\mu\text{m}$ ) were therefore produced by spray-drying and spray-desolvation (Ting et al., 1992).

15 The above observation notwithstanding, several kinds of particulate suspensions were used in the nose, typically to elicit antibodies against the particle-associated antigens.

20 This includes so-called proteosomes comprising gp160 (Lowell et al., 1997) or influenza virus proteins. Another example are particles made from polymerised carbohydrates coated with a lipid (bi)layer.

25 It is important to realise, however, that in any nasal uptake study one should consider and allow for secondary redistribution. For example, the biodistribution of radioactivity from the purified major *Parietaria judaica* allergen after sublingual, oral, and intranasal administration in healthy human volunteers is similar. This is indicative of test material swallowing and absorption in the gastrointestinal tract (Bagnasco et al., 1997). In the intranasal case, transport to the pharynx by mucociliary clearance plays an important role as well, but a relevant fraction of the tracer is retained on the nasal mucosa for up to 48 hours after administration (Bagnasco et al., 1997).

#### 40 **Oral spill-over and the danger of false positive results**

45 Proteins are absorbed in the gastrointestinal tract, albeit in small quantities. For example, ovalbumin (OVA) is absorbed in the stomach as well as from the GI tract into the blood and lymph circulation at levels of 0.007-0.008% and 0.0007-0.002% of applied dose; a higher dose in the latter case leads to relatively higher absorption (Tsume et al., 1996). Stomach absorption supplies nearly exclusively the blood, suggesting different mechanisms and/or routes of absorption between the stomach and the small intestine. OVA association with liposomes can improve the uptake about 50 2 to 3-fold, possibly owing to slower enzymatic degradation of OVA.

Often, the result of nasal and oral immunisation are very similar, suggesting that part of the effect of the former may be due to the spill over of the antigen into gastro-intestinal tract. Data obtained with human adenovirus type 5, used as a vector for heterologous DNA sequences, illustrate this (Flanagan et al., 1997).

#### **Transnasal delivery into the central nervous tissue (CNS)**

The access of substances to the brain is of paramount importance for the treatment of psychiatric and neurologic diseases. Transnasal route of delivery into the CNS was therefore tested for a few selected bioactive molecules.

To date, drug delivery into the CNS tissue by nasal administration has received little attention (Pesechnik & Price, 1996). Wheat-germ agglutinin coupled to horseradish peroxidase was demonstrated to be taken up by the cells of olfactory nerve, resulting in concentration in the olfactory bulb around 0.1% of applied concentration; the underlying principle is probably receptor-mediated endocytosis of WGA and subsequent trans-synaptic, retrograde transfer towards the brain. A similar mechanism is also possible in the case of viral infections in the nose.

For example, an intranasal instillation of vesicular stomatitis virus (VSV), a negative-sense RNA virus, may result in a lethal infection of murine and rat brain (Huneycutt et al, 1994). Within 12 h following intranasal inoculation of VSV, this antigen can be detected in the olfactory nerve layer of the ipsilateral olfactory bulb. Within 3-4 days post-inoculation (p.i.), VSV had disseminated into the glomeruli of the olfactory bulb as well as the anterior olfactory nuclei, ipsilateral to the VSV instillation. Within the glomeruli, VSV antigen is more prevalent in the granule cells than in the mitral cells. Correspondingly, the lateral olfactory tract, where axons of mitral cells course, remain VSV negative throughout 7 days p.i. By 7 days p.i., viral proteins are detected in several additional regions extending to the brainstem. The pattern of VSV immunoreactivity supports the picture of initial infection of the olfactory bulb glomeruli, with subsequent spreads via both ventricular surfaces and retrograde transport within axons of neuromodulatory transmitter systems enervating the olfactory bulb (Huneycutt et al, 1994).

5 Draghia et al. (1995) have demonstrated that it is possible to transfer the *Escherichia coli* lacZ gene in vivo into the central nervous system structures of rats after nasal instillation of replication-defective adenoviral vector AdRSV beta gal. Mitral cells from the olfactory bulb, neurons from the anterior olfactory nucleus, locus coeruleus and  
10 area postrema expressed beta-galactosidase for at least 12 days (Draghia et al., 1995). Parainfluenza type 1 vaccine virus also directly accesses the central nervous system by infecting olfactory neurons (Mori et al., 1996).

15 However, it would be highly desirable to have a convenient and reliable transnasal transport system for the compounds that are capable of and intended to generate a protective immune response without simultaneously generating a variety of adverse side effects. Common types of non-invasive applications, including oral immunisation, often  
20 do not elicit the desired immune response. Many injectible vaccines also do not provide optimum antibody isotype pattern, mainly due to the unnatural route of antigen entry into the body. Transnasal immunisation remains problematic owing to the large size of typical immunogen which is subject to similar restrictions as the transport of  
25 pharmaceutically active compounds across the nasal mucosa.

30 In conclusion, although the prior art has tested various approaches to transnasal delivery it has hitherto failed to provide a convincing principle for convenient and well tolerated transfer of compounds, such as pharmaceutically active substances, immunogens/antigens or allergens, through the nasal barrier, in particular if said  
35 compounds are large. The solution to said technical problem, i.e. the provision of a suitable system, is provided by the embodiments characterised in the claims.

40 Accordingly, the present invention relates to use of a penetrant, suspended or dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-like coating of one or several layers of at least two different substances or two different forms of a substance with the tendency to aggregate, said substances or forms of a  
45 substance differing by at least the factor of 10 in solubility in a preferably aqueous liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or form of the substance or the average diameter of the hetero-aggregates consisting of both said substances or forms of said substance is smaller than the  
50 average diameter of homo-aggregates of the less soluble substance or form of the substance and/or wherein the more soluble component tends to solubilise the



5 penetrating droplet and wherein the content of such component amounts to up to 99  
mol-% of the concentration required to solubilise the droplet or else corresponds to up  
to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is  
10 higher, and/or wherein the elastic deformation energy of the droplet surrounding the  
membrane-like coating is at least 5x lower, more preferably is at least 10x lower and  
ideally is more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains, such droplets then acting as carriers, for the  
15 transnasal administration of pharmaceutically active compounds, antigens, allergens,  
mixture of antigens and/or mixture of allergens.

20 These compounds, antigens or allergens do not cross the nasal mucosa in a practically  
meaningful quantity on their own without causing unacceptable side effects.

25 As regards the above recited values of up to 99%, it is to be noted that values below  
50% of the former relative concentration are particularly useful, with values below 40  
rel-% or even around and below 30 rel-% being even more advantageous, whereas in  
the case of droplets which cannot be solubilised by the more soluble component  
30 relative concentrations which exceed the above mentioned relative concentrations by  
the factor of up to 2 are most preferred.

35 Formulations including the above-referenced penetrants are described in detail in DE  
41 07 152, PCT/EP91/01596, PCT/EP96/04526, and DE 44 47 287, which are  
incorporated herewith by reference. Relevant information useful for penetrant  
manufacturing and loading with various macromolecular actives, which are too big to  
40 permeate through the barrier, is given in patent application PCT/EP98/06750, also  
incorporated herewith by reference.

45 More general information on lipid suspensions can be found in the handbook dealing  
with 'Liposomes' (Gregoriadis, G., ed., CRC Press, Boca Raton, FL, Vols 1-3, 1987), in  
the book 'Liposomes as drug carriers' (Gregoriadis, G., ed., John Wiley & Sons, New  
York, 1988), or in the laboratory manual 'Liposomes. A Practical Approach' (New, R.,  
Oxford-Press, 1989). The properties of phospholipids, which can be used conveniently  
50 to prepare bio-compatible immunopenetrants, are reviewed in 'Phospholipids  
Handbook' (Cevc, G., ed., Dekker, New York, 1995).

5 The manufacturing temperature for said penetrants is normally chosen in the 0°C to 95°C range. Preferably, one works in the temperature range 10-70°C, most frequently at temperatures between 15°C and 45°C, under all circumstances below the temperature at which any important formulation ingredient would undergo an irreversible change in the composition or physical state. These temperatures can be determined by the person skilled in the art using his common general knowledge and the teachings of the various documents cited in this specification. (For reference: the skin temperature is normally around 32°C.) Other temperature ranges are possible, most notably for the systems containing freezable or non-volatile components, cryo- or heat-stabilised formulations, etc.

20 If required to maintain the integrity and the desired properties of individual system components, carrier formulations can be stored in the cold (e.g. at 4°C), with or without associated active agents. It is also possible, and sometimes sensible, to manufacture and store the preparation under an inert atmosphere, e.g. under nitrogen. The shelf-life of carrier formulation, moreover, can be extended by using substances with a small number of double bonds, that is, by a low degree of unsaturation, by choosing peroxide-arm ingredients, by including antioxidants, chelators, and other stabilising agents, or by preparing the agent loaded penetrants *ad hoc* or *in situ*, e.g. from a freeze dried or dry mixture.

35 The term "two forms of a substance" in connection with this invention means two ionization states or salt forms of the same substance, two different complexes of such substance, etc.

40 "Non-invasive administration" or "non-invasive delivery" in this specification denotes application on or transport through the nasal mucosa.

45 "Nasal administration", in the context of this document, refers to applications of test material, whether by direct intranasal intubation, spontaneous sniff of a drop of the test fluid, or an inhalation of the sprayed test-fluid into the nose, independent of precise site of impact or deposition.

50 The term "penetration" in this application describes non-diffusive motion of large entities across a barrier. This process is believed to involve penetrant adaptation to the

5 otherwise confining pores in the barrier, perhaps in association with a transient, selective, and reversible decrease in the barrier resistance.

10 The term "permeation" refers to a diffusion across the semipermeable barrier and is typically driven by the permeant concentration gradient across the barrier.

15 A penetrant, consequently, is an entity comprising a single molecule or an arrangement of molecules too big to permeate through a barrier but capable to cross the barrier owing to the penetrants adaptability to the shape and/or diameter of the otherwise confining passages (pores) of a barrier. This adaptability is seen from the fact, for example, that penetrants more than twice bigger than the pore diameter will cross the bilayer without being fragmented down to the pore size. A permeant, on the other hand, is an entity that can permeate through the semi-permeable barrier, such as the skin. A penetrant in an external field experiences a driving force proportional to the nominal penetrant size and to the applied field, which may occur naturally. Such a force, which on the intact, non-occluded skin is believed to originate from the water concentration gradient across the stratum corneum, can result in a penetrant motion through the barrier, including the skin, if the force is strong enough either to deform the penetrant or else to widen the passages in the barrier sufficiently to elude the problem of size exclusion, or both.

30 A permeant, on the other hand, is a molecule diffusing, or at least capable of diffusion, across the semi-permeable barrier.

40 The above-referenced penetrant is typically an ultra-adaptable entity comprising several components. Said penetrant, in the widest sense of the word, is a supra-macromolecular body that can pass spontaneously through the permeability barrier with pores much smaller than the penetrant diameter, and thus transport material from the application to the destination site on either side of the barrier. In order to meet this goal, the penetrant must adjust its properties, most notably its deformability, to the shape and size of the pores in a barrier. This typically occurs under the influence of a strong driving force or a pressure acting on all molecules in the penetrant. Gradients which do not depend on the penetrant concentration, such as hydration or external electric potential difference across the barrier, were shown to serve this purpose.

5 Lipid aggregates in (quasi)metastable state, and of the nature described above in  
connection with the invention, most often behave as highly adaptable penetrants,  
especially when they have the form of a tiny droplet surrounded by one or a few  
10 membranes (bilayers) (Cevc et al., 1997; Cevc et al., 1998). Owing to membrane  
metastability, unusually high local bilayer curvature can develop at the sites of  
transient, local membrane destabilisation without compromising the overall aggregate  
integrity. From the composition point of view, such ultra-adaptable and self-regulating  
15 vesicles typically consist of a suitably chosen lipid mixture. In order to change  
conventional lipid vesicles, liposomes, into the optimised vesicles (Transfersomes) one  
can add, for example, suitable edge-activators into the aggregate membrane (Cevc et  
al., 1998). Alternatively, molecules which change the system deformability after  
20 complexation with or binding to the basic aggregate ingredient can be used. Often, but  
not necessarily, the activators belong to the class of surfactants below the saturation or  
solubilization concentration, which in the latter case gives rise to mixed micelles  
formation. This is important as solubilised lipids, in the form of mixed lipid micelles, can  
25 cross the pores sufficiently wider than the micelle diameter but are incapable of  
enforcing channel opening in the biological tissues, which can be widened and  
trespassed by the mixed lipid vesicles, however. The postulated reason for this – to  
which the applicant does not wish to be bound - is the much greater aggregation  
30 number of the latter kind of aggregate which translates into the greater sensitivity to  
external, transport-driving gradients, such as the water activity gradient, and which is  
then capable of paying the energetic price for the pore or channel opening in the  
35 barrier.

40 The present invention is, in view of the prior art, particularly surprising since  
ultradeformable lipid vesicles would seem unsuitable for the purpose of transnasal  
delivery taken that they were reported to date to cross barriers, such as skin, only  
under non-occlusive conditions, that is, in the presence of a strong trans-barrier water  
concentration gradient (Cevc et al. 1995; Paul and Cevc, 1995), which is believed not  
45 to exist in the strongly hydrated nasal mucosa.

50 It was unexpectedly found that macromolecules in association with highly adaptable  
penetrants, typically in the form of mixed lipid vesicles, are transported across nasal  
mucosa despite the high water content in this mucosa and in the exhaled air saturated  
with humidity. Concluding from the fact that several successfully tested formulations of  
55

5 such carriers caused no irritation in the nose it is inferred that the aforementioned transport does not rely on damaging the barrier, such damage being the reason for more conventional transport of macromolecules from a solution across the nasal mucosa. Rather than this, it is reasoned (wherein the applicant does not wish to be  
10 bound by theory) that said transport relies on the carrier penetration through the barrier, which should not occur in a very humid surrounding.

15 It is furthermore taught in accordance with the invention that increasing the concentration of the surface active molecules, which can act as permeation enhancers, decreases the efficiency of corresponding protein transport across the nasal mucosa, at least when the solubilisation point of the carriers has been reached. This finding is  
20 unexpected in view of the fact that the art teaches that the bioavailability of nasally administered macromolecules typically gets higher with increasing permeation enhancer concentration.

25 A third unexpected finding is that carrier-mediated delivery of macromolecules across the nasal mucosa can mediate a relatively efficient transport of large molecules into the central nervous system (CNS). The influx is seen relatively soon after the drug  
30 administration into the nasal cavity when the large molecules are associated with the carriers. This could be due to the transport of carrier-associated drugs across the nasal mucosa and subsequent uptake of drug-laden carriers into the olfactory nerve, through which the drug could be carried towards and into the CNS by the retrograde transport;  
35 such transport has already been postulated and was tested with individual molecules (Pasechnik-V; Price-J. Exp. Opin. Invest. Drugs; 5: 1255-1276); the approach was not used, to the best of the applicant's knowledge, in combination with particulates to date.  
40 An alternative explanation would involve the carrier-mediated macromolecular delivery into the peri-nasal lymphatic system, which has been reported to communicate with the central nervous system (Kida-S; Pantazis-A; Weller-RO. Neuropathol. Appl. Neurobiol. 1993; 19: 480-448).  
45

A fourth surprising result achieved in accordance with the present invention is that the  
50 referenced penetrants allow a successful and preferably protective transnasal immunisation with large immunogens. The use of highly adaptable antigen- or immunogen-carriers for the purposes of immunotherapy is expected to or has been shown in accordance with the present invention to provide all the benefits of more  
55

5 conventional nasal vaccinations in addition to the safety and robustness of  
administration. Improved safety would reflect the choice of the non-toxic and non-  
irritating carrier ingredients. Better reproducibility could result from the greater ability of  
10 the specially designed carriers, compared to that of the antigens or immunoadjuvants  
used alone to overcome the nose barrier. Taken the expectation that different carrier  
populations loaded with the individual antigens could be combined into a final multi-  
valent vaccine formulation the capability of invented technology to meet the trend in  
15 immunotherapy is given.

It stands to reason that non-toxic and „gentle“ formulations containing merely bio-  
compatible or natural, body-like ingredients, which protect the body faster and/or better  
20 than the corresponding antigen injections, would be preferred to the latter and would  
have a substantial commercial value.

25 In accordance with the present invention it is recommended to choose the penetrant  
characteristics, especially the deformability, concentration, or composition of the mixed  
lipid aggregates, so as to control the rate or the efficiency of penetrant-mediated  
transport.

30 In the process of optimisation of the formulation and/or administration it may be  
convenient to determine the flux of drug or agent loaded penetrants through the pores  
in a well-defined barrier as a function of suitable driving force or pressure, which act  
35 across the barrier, and then to describe the data by a convenient characteristic curve  
which, in turn, is employed to optimise the formulation or application further.

40 The pharmaceutically acceptable form of the agent may be given in a variety of final  
formulations, optionally, and depending on the purpose of the administration, in  
combination with diverse secondary agents. Such agents will be explained in more  
detail later in the text and may be, for example, bacterial compounds or other  
45 immunomodulations.

50 Furthermore, the present invention relates to the use of a penetrant, suspended or  
dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-  
like coating of one or several layers of at least two different substances or two different  
forms of a substance with the tendency to aggregate, said substances or forms of a  
55

5 substance differing by at least the factor of 10 in solubility in a preferably aqueous,  
liquid medium, such that the average diameter of homo-aggregates of the more soluble  
substance or form of the substance or the average diameter of the hetero-aggregates  
10 consisting of both said substances or forms of said substance is smaller than the  
average diameter of homo-aggregates of the less soluble substance or form of the  
substance and/or wherein the more soluble component tends to solubilise the  
penetrating droplet and wherein the content of such component amounts to up to 99  
15 mol-% of the concentration required to solubilise the droplet or else corresponds to up  
to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is  
higher, and/or wherein the elastic deformation energy of the droplet surrounding the  
membrane-like coating is at least 5x lower, more preferably is at least 10x lower and  
20 ideally is more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains as a carrier for the preparation of a pharmaceutical,  
preferably a vaccine composition for transnasal administration. It is preferred that these  
molecules used on their own do not cross the nasal mucosa in practically useful  
25 quantity without causing unacceptable side effects.

30 The carrier is combined with the pharmaceutically active ingredient prior to the  
administration, e.g. when formulating said pharmaceutical composition. As regards the  
further explanations, description of advantages etc., of this and the following  
embodiments, reference is made to the respective description in connection with the  
35 first embodiment described herein above. It is further to be understood in accordance  
with the present invention that more than one type of antigen, allergen or  
pharmaceutically active ingredient or combinations thereof may be formulated into said  
pharmaceutical composition.

40 Additionally, the present invention relates to the use of a penetrant, suspended or  
dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-  
like coating of one or several layers of at least two different substances or two different  
45 forms of a substance with the tendency to aggregate, said substances or forms of a  
substance differing by at least the factor of 10 in solubility in a preferably aqueous,  
liquid medium, such that the average diameter of homo-aggregates of the more soluble  
substance or form of the substance or the average diameter of the hetero-aggregates  
50 consisting of both said substances or forms of said substance is smaller than the  
average diameter of homo-aggregates of the less soluble substance or form of the

5 substance and/or wherein the more soluble component tends to solubilise the  
penetrating droplet and wherein the content of such component amounts to up to 99  
mol-% of the concentration required to solubilise the droplet or else corresponds to up  
10 to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is  
higher, and/or wherein the elastic deformation energy of the droplet surrounding  
membrane-like coating is at least 5x lower, more preferably is at least 10x lower and  
ideally is more than 10x lower than that of the red blood cells or of the phospholipid  
15 bilayers with fluid aliphatic chains in combination with a pharmaceutically active  
ingredient or an allergen or an antigen for the preparation of a transnasally  
administerable pharmaceutical composition for the treatment of infective diseases,  
endocrine disorders, preferably hypopituitarism, diabetes, hyperthyroidism, thyroiditis,  
20 most preferably Hashimoto's thyroiditis, subacute thyroiditis; adrenal disorders,  
preferably Addison's disease, secondary adrenal insufficiency, Cushing's syndrome;  
gastrointestinal disorders, preferably Crohn's disease, colitis; hemorrhagic diseases,  
preferably hemophilia, leukopenia, hypereosinophilic syndrome; musculoskeletal and  
25 connective tissue disorders, preferably rheumatoid arthritis, Sjögren's syndrome,  
Bechet's syndrome, lupus, scleroderma, polymyositis/dermatomyositis, polymyalgia  
rheumatica and temporal arthritis, polyarteriosis nodosa, Wegener's granulomatosis,  
mixed connective tissue disorder, ankylosing spondylitis, psoriatic arthritis,  
30 osteoarthritis, Paget's disease, sciatica, bursitis, tendonitis or tenosynovitis,  
epicondylitis, fibromyalgia, eosinophilic fasciitis; neurological disorders, preferably pain,  
singultus, vertigo, seizure disorders, sleep disorders, transient ischemic attacks, spinal  
cord injury, demyelinating diseases, nerve root disorders, myasthenia gravis;  
35 psychiatric disorders, preferably drug dependence, neuroses, mood disorders,  
schizophrenic disorders, delusional disorders; for oncological purposes and/or for  
treatment in the field of gynecology, preferably for the treatment of dysmenorrhea,  
menopause, chronic anovulation, premature ovarian failure, endometriosis, infertility;  
40 and/or for treatment in the field of immunology, preferably transplant rejection,  
hyposensitisation, allergen immunotherapy or prophylactic vaccination.

45 The term „allergen“ is used in this invention to describe materials of endogenous or  
xenogenic, e.g. animal or plant, origin which result in an undesired immune response  
of the body exposed to such an allergen, often resulting in an acute hypersensitivity  
50 reaction. Allergising microbes or parts thereof (e.g. of mite), parts of plants (e.g.  
pollen) or animal (e.g. hair and skin debris), but also man made and inorganic



5 substances belong to this group. On the other hand, nearly any part of the human body, if incorrectly processed by or exposed to the body's immune system, can result in an auto-immune response and lead to the allergic reaction to such a substance. In the narrower interpretation, used when so stated, an allergen is a substance, a group, 10 or an arrangement of substances causing immediate hypersensitivity reactions in the body that could be diminished, or even eliminated, by an immunotherapy, whether done non-invasively through the nasal mucosa or not.

15 An „antigen“ is a part of a pathogen or an allergen in its natural form or after fragmentation or derivatisation. More generally, the word antigen denotes a macromolecule or a fragment thereof, any haptenic moiety (for example, a simple carbohydrate, complex carbohydrate, polysaccharide, deoxyribonucleic acid), in short, 20 any molecule recognized by a body's antibody repertoire and possibly capable of antibody induction when administered in the system. A macromolecular antigen is defined as an antigen that is known to or believed to cross spontaneously the nasal barrier only in quantity too small for the desired practical purpose. Thus, 25 macromolecules are molecules that, on their own, do not cross the nasal mucosa in practically useful quantity without causing unacceptable side effects.

30 The term "a mixture of antigens" or "a mixture of allergens" means, in accordance with the present invention, the combination of at least two antigens and/or allergens. It is envisaged that also mixtures of antigens and allergens can be used according to the 35 present invention.

40 Furthermore, the present invention relates to a pharmaceutical composition for transnasal administration comprising a carrier which is a penetrant, suspended or dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-like coating of one or several layers of at least two different substances or two different forms of a substance with the tendency to aggregate, said substances or forms of a 45 substance differing by at least the factor of 10 in solubility in a preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or form of the substance or the average diameter of the hetero-aggregates consisting of both said substances or forms of said substance is smaller than the 50 average diameter of homo-aggregates of the less soluble substance or form of the substance and/or wherein the more soluble component tends to solubilise the

5 penetrating droplet and wherein the content of such component amounts to up to 99  
mol-% of the concentration required to solubilise the droplet or else corresponds to up  
to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is  
10 higher, and/or wherein the elastic deformation energy of the droplet surrounding the  
membrane-like coating is at least 5x lower, more preferably is at least 10x lower and  
ideally is more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains and a pharmaceutically active ingredient.

15 In a preferred embodiment of the use or the pharmaceutical composition of the present  
invention the pharmaceutically active ingredient is an adrenocorticostaticum, an  
adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an  
20 anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum,  
antiartheroscleroticum, antiasthmaticum and/or bronchospasmolyticum, an antibioticum,  
an anti-infective agent, an antidepressivum and/or antipsychoticum, an antidiabeticum,  
an antidot, an antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or  
25 anticholinergicum, an enzyme, a coenzyme or the corresponding enzyme inhibitor, an  
antihistaminicum (and combinations thereof) or antihypertonicum, an antihypotonicum,  
an anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus  
30 Alzheimer or Morbus Parkinson, an agent for ACS therapy, an antiphlogisticum,  
antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a  
respiratory stimulant, a broncholyticum, cardiotonicum, chemotherapeuticum, a  
coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid,  
35 an anti-flew agent, a haemostaticum, hypnoticum, an immunoglobuline or its fragment  
or any other immunologically active substance, such as an immunomodulator, a  
bioactive carbohydrate (derivative), a contraceptive, an anti-migraine agent, a  
corticosteroid, a muscle relaxant, a narcoticum, a neurotherapeuticum, a  
40 (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide (derivative), an  
opiate, an opthalmicum, (para)-sympaticomimeticum or (para)sympathicolyticum, a  
protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant,  
45 rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum,  
tuberculostaticum, an urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a  
wound-healing substance, an alcohol abuse preparation, an anticonvulsant, an  
antineoplastic, an antirheumatic, an appetite suppressant, a biological response  
50 modifier, a blood modifier, a bone metabolism regulator, a cardioprotective agent, a  
cardiovascular agent, a central nervous system stimulant, an enzyme, an agent for

5       erectile dysfunction therapy, a fertility agent, a gastrointestinal agent, a gout  
preparation, a hormone, an agent for hypercalcemia management, an agent for  
hypocalcemia management, an immunosuppressive, a migraine preparation, a motion  
10       sickness product, an agent for multiple sclerosis management, a muscle relaxant, a  
nutritional, an ophthalmic preparation, an osteoporosis preparation, an otic preparation,  
a parasympatholytic, a parasympathomimetic, a prostaglandin, a psychotherapeutic  
agent, a respiratory agent, a sedative & hyponotic, a skin & mucous membrane agent,  
15       a smoking cessation aid, a sympatholytic, a tremor preparation, a urinary tract agent, a  
vaginal preparation, a vertigo agent, an inhibitor (antagonist), or any other  
immunologically active substance (such as an immunomodulator, e.g., bacterial  
extracts or cell wall components like cholera toxin, heat labile toxin,  
20       monophosphoryllipid A, or cytokine inducing agents or hormones like thymosin,  
thymulin, thymopoietin, or phytoimmunostimulants like extracts from Echinacea root,  
wild indigo root, white cedar leave tips, or synthetic immunomodulators like quinoline  
derivatives, synthetic peptides, pyrimidine, lipopeptides, or cytokines or  
25       immunosuppressants, and signal transduction inhibitors like cyclosporin A, FK506,  
FTY720, rapamycin), or a promotor (agonist) of the activity of any of above mentioned  
agents, or any combination of said active substances. It is preferred that said active  
30       ingredient does not itself cross the nasal mucosa in practically meaningful quantity  
without unacceptable side effects.

35       In another preferred embodiment of the use or the pharmaceutical composition of the  
present invention the antigen is derived from a pathogen.

40       In the context of this invention, the term „pathogen“ refers to an entity which through its  
presence in or on the body leads to or promotes a pathological state which, in  
principle, is amenable to or could profit from a preventive, curative or adjuvant  
immunotherapy.

45       In a most preferred embodiment of the use or the pharmaceutical composition of the  
invention said pathogen belongs to the class of extracellular bacteria, including pus-  
forming cocci, such as *Staphylococcus* and *Streptococcus*, gram-negative bacteria,  
50       such as *Meningococcus* and *Gonococcus* species, species of *Neisseria*, gram negative  
bacteria, including enteric organisms such as *E. coli*, *Salmonella*, *Shigella*,  
*Pseudomonas*, *Diphtheria*, *Bordetella Pertussis*, and gram-positive bacteria (e.g.

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5 *Bacillus pestis*, *BCG*), particularly anaerobes, such as the *Clostridium* species (e.g. *Clostridium tetani*, *Clostridium perfringens*, *Clostridium novyi*, *Clostridium septicum*),  
bacteria and viruses, which survive and replicate within host cells, comprising  
10 mycobacteria (e.g. *M. tuberculosis*) and *Listeria monocytogenes*, retro- and  
adenoviruses, including hepatitis virus, (human) immunodeficiency virus, herpes  
viruses, small-pox (chicken-pox), influenza, measles, mumps and polio viruses,  
15 cytomegalovirus, rhinovirus, etc., and fungi prospering inside host cells, parasites  
including animal parasites, such as protozoa and helminths, and ectoparasites, such  
as ticks and mites, or *Brucella* species (e.g. *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*,  
*B. neotomae*, *B. ovis*), the causative agent for cholera (e.g. *Vibrio cholerae*),  
20 *Haemophilus* species like *H. actinomycentemcomitans*, *H. pleuropneumoniae*, as well  
as pathogens triggering paratyphoid, plague, rabies, tetanus and rubella diseases;  
eukaryotic cells or their parts that cause various neoplasiae, auto-immune diseases  
and other pathological states of the animal or human body, which do not necessarily  
25 result from microbial infections, also belong in this group.

It is most preferred that the antigen, preferably the pathogen, is used in a purified, or  
even better in a pure form.

30 Pathogens causing major infective diseases such as hepatitis virus, (human)  
immunodeficiency virus, herpes viruses, small-pox (chicken-pox), influenza, measles,  
mumps and polio viruses, cytomegalovirus, rhinovirus, etc., and fungi prospering inside  
35 host cells, a parasite including animal parasites, such as protozoa and helminths, and  
ectoparasites, such as ticks and mites, or *Brucella* species, or the causative agent for  
cholera, *Haemophilus* species, as well as pathogens triggering paratyphoid, plague,  
40 rabies, tetanus and rubella diseases are particularly preferred as are eukaryotic cells or  
their parts that cause various neoplasiae, auto-immune diseases and other  
pathological states of the animal or human body, which do not necessarily result from  
microbial infections.

45 In another preferred embodiment of the use or the pharmaceutical composition of the  
invention the allergen is of xenogenic or endogenic origin, derived from a  
50 microorganism, an animal or a plant, or belonging to the group of man made and/or  
irritating inorganic substances, or to such parts or components of the human body  
which were incorrectly processed by or exposed to the body immune system.

5 In further preferred embodiment of the use or the pharmaceutical composition of the present invention the allergen belongs to the class of the inhalation allergens, including but not limited to various pollen, spores, bits of animal hair, skin, feather, natural and synthetic textiles, wheat, (house) dust, including mite; furthermore, food and drug  
10 allergens; contact allergens; injection, invasion or depot allergens, such as various (gastrointestine-resident) worms, echinococci, trichines, etc., or is a part of implantation material.

15 In an additional preferred embodiment of the use or the pharmaceutical composition of the present invention said pharmaceutical composition comprises a compound which releases or induces cytokine or anti-cytokine activity or exerts such an activity itself.

20 The term „cytokine“, as used in the present invention, denotes cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, with all subtypes, such as IL-1 $\alpha$  and IL-1 $\beta$ , tumour necrosis factor (TNF),  
25 transforming growth factor (TGF- $\beta$  and - $\alpha$ ), Type I and II interferons (IFN- $\alpha$ 1, IFN- $\alpha$ 2, (IFN- $\omega$ ), IFN- $\beta$ , IFN- $\gamma$ ), migration inhibitory factor, MIF, c-kit ligand, granulocyte macrophage colony stimulating factor (GM-CSF), monocyte macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF),  
30 chemokines, etc., as well as all functional derivatives of any of these molecules.

35 Cytokines that mediate natural immunity particularly well include type I interferons (IFN- $\alpha$  and IFN- $\beta$ ), tumour necrosis factor (TNF), interleukin-1 (IL-1 $\alpha$  and IL-1 $\beta$ ), interleukin-6 (IL-6) and leukocytes attracting and activating chemokines. Antiproliferative (e.g. with IFN-s), pro-inflammatory (e.g. with TNF, IL-1) or co-  
40 stimulatory (e.g. with IL-6) action, amongst other, may be generated by transnasal administration of the pharmaceutical composition described in accordance with the present invention. Cytokines which best mediate lymphocyte activation, growth and differentiation include interleukin 2 (IL-2), interleukin-4 (IL-4) and transforming growth  
45 factor (TGF). Such cytokines, consequently, not only can affect target growth but, moreover, influence the activation of, and thus the production of other cytokines by, the cells which finally may play a role in therapeutic or prophylactic action.

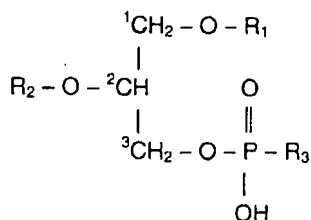
Cytokines that mediate the immune-mediated inflammation which heavily relies on the cell-mediated response are interferon-gamma (IFN- $\gamma$ ), lymphotoxin (TNF- $\beta$ ), interleukin-10 (IL-10), interleukin-5 (IL-5), interleukin-12 (IL-12) and, probably, migration inhibition factor. Leukocyte growth and differentiation are most affected by interleukin-3 (IL-3), c-kit ligand, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage or granulocyte colony stimulating factor (M-CSF or G-CSF) and interleukin-7 (IL-7).

It is preferred to select the compound displaying cytokine activity amongst IL-4, IL-2, TGF, IL-6, TNF, IL-1 $\alpha$  and IL-1 $\beta$ , a type I interferon, amongst which IFN-alpha or IFN- $\beta$  are most preferred, IL-12, IFN- $\gamma$ , TNF- $\beta$ , IL-5 or IL-10.

In another preferred embodiment said compound with anti-cytokine activity is an anti-cytokine antibody or the corresponding active fragment, a derivative, or an analogue thereof.

In another preferred embodiment of the use or of the pharmaceutical composition of the present invention, the compound displaying or inducing cytokine or anti-cytokine activity and the pharmaceutically active ingredient or antigen or allergen are associated with the penetrant, e.g. in the form of a complex, hetero-aggregate, via encapsulation etc.

In an additional preferred embodiment of the use or of the pharmaceutical composition of the present invention the less soluble self-aggregating molecule is a lipid, preferably a polar lipid, and the more soluble component is a surfactant or some more soluble form of the polar/basic lipid. The former ingredient, typically, stems from a biological source or is a corresponding synthetic lipid or any of its modifications. Such lipid often belongs to the class of phospholipids with the chemical formula



5 where  $R_1$  and  $R_2$  is an aliphatic chain, typically a  $C_{10-20}$ -acyl, or -alkyl or a partly unsaturated fatty acid residue, in particular, an oleoyl-, palmitoleoyl-, elaidoyl-, linoleyl-, linolenyl-, linolenoyl-, arachidoyl-, vacciny-, lauroyl-, myristoyl-, palmitoyl-, or stearoyl chain, and where  $R_3$  is hydrogen, 2-trimethylamino-1-ethyl, 2-amino-1-ethyl,  
10  $C_{1-4}$ -alkyl,  $C_{1-5}$ -alkyl substituted with carboxy,  $C_{2-5}$ -alkyl substituted with hydroxy,  $C_{2-5}$ -alkyl substituted with carboxy and hydroxy, or  $C_{2-5}$ -alkyl substituted with carboxy and amino, inositol, sphingosine, or salts of said substances, said lipid comprising also glycerides, isoprenoid lipids, steroids, sterines or sterols, of sulphur- or carbohydrate-containing lipids, or any other bilayer forming lipids, and preferably is selected from the group of phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins or other sphingophospholipids, glycosphingolipids (including cerebroside,  
20 ceramidepolyhexosides, sulphatides, sphingoplasmalogens), gangliosides or other glycolipids or synthetic lipids, in particular with corresponding sphingosine derivatives, or any other glycolipids, whereby two similar or different chains can be esterified to the backbone (as in diacyl and dialkenoyl compound) or be attached to the backbone with ether bonds, as in dialkyl-lipids, or belong to the backbone as in sphingolipids.

30 The surfactant used, normally, is nonionic, zwitterionic, anionic or cationic, especially a fatty-acid or -alcohol, an alkyl-tri/di/methyl-ammonium salt, an alkylsulphate salt, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurodeoxycholate, taurocholate, etc., an acyl- or alkanoyl-dimethyl-aminoxide, esp. a  
35 dodecyl- dimethyl-aminoxide, an alkyl- or alkanoyl-N-methylglucamide, N-alkyl-N,N-dimethylglycine, 3-(acyldimethylammonio)-alkanesulphonate, N-acyl-sulphobetaine, a polyethylene-glycol-octylphenyl ether, esp. a nonaethylene-glycol-octylphenyl ether, a polyethylene-acyl ether, esp. a nonaethylen-dodecyl ether, a polyethylene-glycol-isoacyl ether, esp. a octaethylene-glycol-isotridecyl ether, polyethylene-acyl ether, esp. octaethylenedodecyl ether, polyethylene-glycol-sorbitane-acyl ester, such as polyethylenglykol-20-monolaurate (Tween 20) or polyethylenglykol-20-sorbitan-  
40 monooleate (Tween 80), a polyhydroxyethylene-acyl ether, esp. polyhydroxyethylene-lauryl-, -myristoyl-, -cetylstearyl-, or -oleoyl ether, as in polyhydroxyethylene-4 or 6 or 8 or 10 or 12, etc., -lauryl ether (as in Brij series), or in the corresponding ester, e.g. of polyhydroxyethylen-8-stearate (Myrj 45), myristate-, -laurate, linoleate-, linolenate-,  
50 palmitoleate- or -oleate type, or in polyethoxylated castor oil 40, a sorbitane-monoalkylate (e.g. in Arlacel or Span), esp. sorbitane-monolaurate, -myristate,

5 -linoleate, -linolenate-, -palmitoleate- or -oleate, an acyl- or alkanoyl-N-methylglucamide, esp. in or decanoyl- or dodecanoyl-N-methylglucamide, an alkyl-sulphate (salt), e.g. in lauryl-, myristoyl, palmitoyl, oleoyl-, palmitoleoyl-, linolenyl-, linoleoyl-, vaccinyl-, or elaidoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, a fatty acid salt, with similar  
10 preference for aliphatic chains as given above, a lysophospholipid, such as n-octadecylene(=oleoyl)-glycerophosphatidic acid, -phosphorylglycerol, or -phosphorylserine, n-acyl-, e.g. lauryl, myristoyl, palmitoyl, oleoyl-, palmitoleoyl-,  
15 elaidyl-, vaccinyl-, linoleyl-, linolenyl-glycero-phosphatidic acid, -phosphorylglycerol, or -phosphorylserine, or a corresponding short, double chain phospholipid, such dodecyl-phosphatidylcholine, or else is a surface-active polypeptide. It is important to realise,  
20 however, that complexes of polar lipids with other amphipats often can take the role of surfactants in the coating of a carrier and that different ionisation or salt forms of the polar lipids may differ widely in their properties. It therefore stands to reason that two different physicochemical states of the same (polar) lipid mixed together in a  
25 membrane may produce a highly deformable carrier satisfying the conditions of this invention.

30 In an additional preferred embodiment of the use or of the pharmaceutical composition of the present invention, the more soluble component is an agent to be transported across the barrier, said agent having a tendency to form common large structures with the less soluble component(s) of the penetrant, typically in the form of a physical or a  
35 chemical complex.

40 In a further preferred use or pharmaceutical composition of the invention, the more soluble component tends to solubilise the penetrating droplet and is present in concentration not exceeding 99 mol% of the concentration required to disintegrate the droplet or, alternatively, not exceeding 99 mol% of the saturating concentration in the unsolubilised droplet, whichever is higher, values below 50% of the former relative  
45 concentration being particularly useful, with values below 40 rel-% or even around and below 30 rel-% being even more advantageous, whereas in the case of droplets which cannot be solubilised by the more soluble component relative concentrations which  
50 exceed the above mentioned relative concentrations by the factor of up to 2 are most preferred.



5 In a different preferred embodiment of the use or of the pharmaceutical composition of the invention, the less soluble penetrant component is a lipid, preferably a polar lipid and the more soluble component is a surfactant or a surfactant-like molecule or else such form of polar lipid which is sufficiently soluble for the purpose of this invention.

10 In another preferred embodiment of the use or of the pharmaceutical composition of the present invention, the average penetrant diameter is between 25 nm and 500 nm, preferably between 30 nm and 250 nm, even more preferably between 35 nm and 200 nm and particularly preferably between 40 nm and 150 nm.

15 In a different preferred embodiment of the use or of the pharmaceutical composition of the present invention the penetrant concentration in the formulation for the use in the human or animal nose is 0.001 weight-% (w-%) to 20 w-% of total dry mass in the formulation, in particular between 0.01 w-% and 15 w-%, more preferably between 0.1 w-% and 12.5 w-% and most preferred between 0.5 w-% and 10 w-%.

20 In a further preferred embodiment of the use or of the pharmaceutical composition of the present invention the supporting medium, e.g. a buffer, is selected to be a biocompatible solution with an osmotic activity similar to that of a monovalent electrolyte with a concentration range between 1 mM and 500 mM, more preferably between 10 mM and 400 mM, even more preferably between 50 mM and 300 mM, and most preferably between 100 mM and 200 mM or else such solution that affords  
25 practically sufficient penetrant stability combined with practically sufficient transport rate across the barrier. The term "practically sufficient penetrant stability" means that the penetrant stability meets the reasonable product quality criteria. The term "practically sufficient transport rate" means that enough drug is transported through the  
30 barrier without using unreasonably large application volume or time. Said sufficient penetrant stability combined with sufficient transport rate across the barrier can be determined by the person skilled in the art without undue experimentation.

35 In another preferred embodiment of the use or of the pharmaceutical composition of the present invention, the relative drug or agent concentration is between 0.001 w-% and 40 w-% of total penetrant mass, in particular between 0.01 w-% and 30 w-%, even better between 0.1 w-% and 25 w-% and most preferably between 0.5 w-% and  
40 15 w-%.

5 In one further preferred embodiment of the use or of the pharmaceutical composition of the present invention the medium supporting the drugs and carriers is a biocompatible buffer with pH value between 4 and 10, more frequently between 5 and 9 and most  
10 often between 6 and 8.

15 In another preferred embodiment of the use or of the pharmaceutical composition of the present invention additives are included in said composition to reduce the system sensitivity to chemical, biological or ambient stress, including anti-oxidants, antagonists of undesired enzyme action, cryo-preservants, microbicides, etc., or else modulators of physically important system properties, such as formulation viscosity, etc.

20 In a different preferred embodiment of the use or of the pharmaceutical composition of the present invention the relative drug or agent dose to be administered non-invasively through the nose by means of highly adaptable carriers is chosen to be between 0.1x  
25 and 500x, more often between 0.5x and 250x, and even more preferably between 1x and 100x different from the corresponding drug or agent dose that would have to be injected to achieve the desired biological effects. Again, the latter dose can be  
30 determined by the person skilled in the art without undue experimentation and on the basis of his common general knowledge.

35 In another preferred embodiment of the use or of the pharmaceutical composition of the present invention the applied penetrant dose is between 0.01 mg and 15 mg per nostril, even more often is in the range 0.1 mg and 10 mg per nostril, and preferably is between 0.5 mg and 5 mg per nostril.

40 The efficiency of administration and the biological effects of the agent or drug chosen, consequently, can be controlled by using different application volumes. Various metered delivery devices can be used for the purpose.

45 Accordingly, in an additional preferred embodiment of the use or of the pharmaceutical composition of the present invention said formulation is administered using a metered  
50 delivery device.

5 In one further preferred embodiment of the use or of the pharmaceutical composition of the present invention different application volumes are selected to control the efficiency of administration and the biological effects of the chosen agent or drug.

10 In a different preferred embodiment of the use or of the pharmaceutical composition of the present invention the penetrants in suspension are loaded with the drugs or agents within 24 hours prior to the formulation administration, preferably 360 min, more preferably 60 min and even more preferably 30 min before the resulting formulation is  
15 administrated in the nose. This embodiment is expected to improve the formulation stability, loading efficiency, the release kinetics, ease of use, compliance, etc.

20 In another preferred embodiment of the use or of the pharmaceutical composition of the present invention the, delivery device is loaded at the treatment site.

25 In a further preferred embodiment of the use or of the pharmaceutical composition of the present invention the delivery device is loaded separately with penetrants and the molecules, particularly biological agents, to be associated therewith.

30 In one further preferred embodiment of the use of the present invention wherein the pharmaceutically active ingredient is for administration to the nervous system.

35 The term "administration" in connection with this embodiment means that the pharmaceutical composition is transnasally applied, but the target site of the active ingredient is the nervous system, preferably the CNS and most preferably the brain. The possibility to use nasal application of the highly adaptable, drug-loaded penetrants  
40 in the nose to mediate a practically useful transfer of the drug across the barrier can thus be exploited to transport a meaningful amount of the drug, and to create a significant concentration of such drug, in the central nervous system or some other adjacent tissue, such as the eye.

45 In another preferred embodiment of the invention, the pharmaceutical composition of the invention is a vaccine.

50 Said vaccine can be used for therapeutic or prophylactic vaccination.

5 The term „(therapeutic) vaccination“ in the context of this invention describes any kind of therapeutic immunisation, whether done after the disease has been already established, to improve a clinical situation, or else for the purpose of preventing a disease. Such a vaccination can involve single or repeated administration(s) of the vaccine of the invention. Therapeutic vaccination will either prevent a pathological situation and/or improve a clinical situation. When applied as a preventive agent, it will generally result in a protective immune response.

15 Immunisation denotes any kind of provoking an immune response, irrespective of whether said response is therapeutic or non-therapeutic.

20 An „antibody“ or an „immunoglobulin“ denotes an IgA, IgD, IgE, IgG, or IgM, including all subtypes, such as IgA1 and IgA2, IgG1, IgG2, IgG3, IgG4. Their „derivatives“ include chemical, biochemical and otherwise obtainable derivatives, such as genetically engineered antibody derivatives. Fragments include e.g. single chain fragments, Fc-, Fab- F(ab')<sub>2</sub>- and other parts of Ig-s, independent of whether they are of endogenous, xenogenic, (semi)synthetic or recombinant origin. Also comprised by the invention are complexes of two or more of the above-recited antibodies, derivatives or fragments.

30 The term „immunogen“ denotes a hapten coupled to an immunological carrier or an antigen, free or associated with a carrier, which is capable of inducing an immune response.

40 „Immuno-tolerance“ denotes the lack or, more generally, the reduction of an undesired immune response to an antigen.

Th1 (T-helper cell type I) related antibodies include IgG2a, IgG2b and IgG3.

45 Th2 (T-helper cell type II) related antibodies comprise the classes of IgG1, IgG4 and IgE.

50 As has been indicated above, the successful immunisation with the vaccine of the invention through the nose is a significant step forward in the design of conveniently administrable vaccines that (a) are highly efficient over a wide range of immunogens of

5 varying size and properties; (b) can be formulated together with certain cytokines, compounds that mediate cytokine activity or compounds that antagonize cytokine activity in order to specifically direct the corresponding immune response or to  
10 augment or suppress the same as may be desired; (c) do not depend on the perturbing injection by a needle; and (d) cause no irritating side effects. In addition, with the vaccine of the invention, successful tolerogenisation may be achieved.

15 It has inter alia been found in accordance with the present invention that

- Tween-SPC micelles give protection significantly below that of the vaccine of the present invention, suggesting that the small size of the carrier or the presence of surfactants alone does not suffice for a successful immunisation;
- orally administered immuno-carriers create lower specific antibody titers than the transnasally administered vaccine of the invention, as determined on the basis of absorbance measurements;
- the transnasal vaccine of the invention gives rise to higher specific IgG1 and IgG2 titers in the blood and to comparable IgG2a and IgM titers as compared to mixed micelles; all titers were, on top of this, higher than those generated by immunisation with SPC:cholesterol (1:1) liposomes.

30 When the transnasal vaccine of the invention is formulated together with a cytokine or an immunoadjuvant it is advantageous to use (blends of) bacterial extracts. Specific examples given in this application include monophosphoryl lipid A (MPL) and IL-12 or GM-CSF and IL-4. In principle, however, the vaccine of the invention may be  
35 formulated or applied together with any of the compounds mediating, inducing or displaying cytokine activity or with antagonists thereto that have been recited herein above.

40 It is preferred that the vaccine of the invention further comprises a pathogen extract or a compound from a pathogen or a fragment or a derivative thereof.

45 Most preferably, said pathogen extract or compound is selected from hepatitis virus, (human) immunodeficiency virus, herpes viruses, small-pox (chicken-pox), influenza, measles, mumps or polio viruses, cytomegalovirus, rhinovirus, etc., or fungi prospering  
50 inside host cells, a parasite including animal parasites, such as protozoa and helminths, and ectoparasites, such as ticks and mites, or *Brucella* species, including

the causative agent for cholera (e.g. *Vibrio cholerae*), *Haemophilus* species, as well as pathogens triggering paratyphoid, plague, rabies, tetanus or rubella diseases.

It is additionally preferred that said vaccine further comprises an adjuvant.

The term „adjuvant“ is used here to describe any substance which supports, augments, stimulates, activates, potentiates or modulates the desired immune response of either cellular or humoral type, specifically in the case of a prophylactic treatment by increasing the antigen specific immune response of any kind and in the case of therapeutic treatment often by supporting cell-mediated immunity. This can be achieved by the addition of suitable cytokines, their blends or suitable agonists and antagonists. The class of immunoadjuvants which indirectly contribute to the useful cytokine pool includes small chemical entities with an allergenic potential, such as certain allergenic (metal) ions, including but not limited to LiCl, HgCl<sub>2</sub>, molybdenum, acids, bases and other irritating compounds, such as dicyclohexylmethane-4,4'-diisocyanate, ditrocarb (diethyldithiocarbamate), 2,4-dinitrochlorobenzene, isoprinosine, isophorone-diisocyanate, levamisole, (phenyl)oxazolone and alike, Swansonine, sizofran, phthalic anhydride, thymopentin, (fatty) alcohols, (fatty) amines, (fatty) ethers, ricin, or other suitable amphiphiles, many surfactants and chemical permeation enhancers, as well as derivatives or combinations thereof; furthermore, (low molecular weight) fragments of or derivatives from microbes, including lipopolysaccharides (such as LPS), cord-factor (trehalose-dimycolate) and other (poly)saccharides or (poly)peptides attached to membranes, used in sufficient quantity, acetylmuramyl-alanyl-isoglutamin, and larger fragments of microbes, including bacterial exo- and endotoxins, or enterotoxins, such as cholera toxin and the heat labile toxin of *E. coli*, and their macromolecular fragments, such as A-chain derivatives, most, if not all, of which seem to possess ADP-ribosylating activity, the high potency immunoadjuvant LT holotoxin, etc., cell-wall skeleton, attenuated bacteria, such as BCG, etc. Less established examples include clostridial toxin, purified protein derivative of *M. tuberculosis*, LT-R192G, Fibronectin-binding protein I of *Streptococcus pyogenes*, outer membrane protein of group B *Neisseria meningitidis* (GBOMP), various other peptidoglycans, etc. Immunoadjuvants, in other words, include molecules that alter the uptake or presentation of antigens, activate or increase the proliferation of antigen specific lymphocytes, or interfere with the dominant control mechanism in the immune response, not just in the nose but also in the other

immunocompetent tissues. (The mucosal adjuvant activity of ADP-ribosylating bacterial enterotoxins is a well established and known example for this.) On the other hand, molecules which change the (relative) concentrations of cytokines or other immunoadjuvants, such as anti-immunoadjuvant antibodies or other agonists or antagonists of immunoadjuvants, also are immunoadjuvants in the sense of this invention. The same is true for molecules which affect lymphocyte homing, such as various selectins (LECAMS, e.g. various CD62-s), GlyCAM-1, MadCAM-1, VCAM-1, ICAM-1, hyaluronate, etc., and other chemokines, such as RANTES or MCP-1. Endogenous group of immunoadjuvants furthermore comprises histamines, transfer factor, tuftsin, etc. As many of the above mentioned immunoadjuvants do not have sufficient potency to ensure the desired effect after the non-invasive immunisation at too low, and sometimes too high, concentration or on their own, the functional definition of an adjuvant used in this work includes a fortiori sufficient and such modulation of cytokine concentration and distribution pattern in the body that results in mounting the desired therapeutic or prophylactic immune response. If required to gain clarity said modulation and its extent must be determined in a dedicated experiment, in which the specific cytokine levels are determined, using methods known to the person skilled in the field.

In a further preferred embodiment of the vaccine of the invention, said adjuvant is lipopolysaccharide, such as lipid A or a derivative or modification thereof, such as monophosphoryl lipid A, or its analogue, such as a fatty derivative of saccharose, cord-factor (trehalose-dimycolate), muramyl dipeptide, or another (poly)saccharide or (poly)peptide identical to or resembling an immunologically active part of a membrane of a microorganism; an extract of a microorganism, including bacterial exo- and endotoxins, preferably cholera toxin or the heat labile toxin of *E. coli*, an A-chain derivative, a component with an ADP-ribosylating activity, a peptidoglycane, a clostridial toxin, an LT halotoxin, purified protein derivative of *M. tuberculosis*, LT-R192G, Fibronectin-binding protein I of *Streptococcus pyrogenes*, or outer membrane protein of group B *Neisseria meningitidis* (GBOMP), bacterial or viral nucleic acids, such as oligonucleotides comprising unmethylated CpG dinucleotides.

It is most preferred that the vaccine of the invention comprises a blend of MPL and IL-12 or GM-CSF and IL-4, when pure cytokines and their inducers are used.

5 In a different preferred embodiment of the vaccine of the present invention the relative immunogen/antigen dose to be administered non-invasively through the nose by means of highly adaptable carriers is chosen to be between 0.01x and 100x, more often between 0.05x and 75x, and even more preferably between 0.1x and 50x different from the corresponding immunogen/antigen that would have to be injected to achieve the desired biological effect. Again, the latter dose can be determined by the person skilled in the art without undue experimentation and on the basis of his common general knowledge.

15 It is further preferred in accordance with the invention that in said vaccine the concentration of the transnasally administered adjuvant is between 10x lower and up to 1000x higher than used with the corresponding subcutaneously injected formulations employing similar antigen, the transnasally administered immunoadjuvant concentration more often differing from the injected immunoadjuvant concentration by the factor between 0.5 and 100, or better, by the factor between 1 and 50, and best between 2 and 25.

20 The invention also relates to a container comprising the pharmaceutical composition recited herein-above. The unit dosage may be determined according to the desired application.

30 Furthermore, the invention additionally relates to a package comprising at least one container comprising the pharmaceutical composition as described above. The package of the invention can comprise one, two, three, four or more vials/units of the pharmaceutical composition of the invention.

40 The invention finally relates to methods of treating a patient in need thereof comprising transnasally administering any of the above recited pharmaceutical compositions.

45 The present invention further relates to a method for generating a protective or tolerogenic immune response on a mammal by vaccinating said mammal with a vaccine as described above.

50 In a preferred embodiment of the method according to the present invention different administration volumes are selected to control the applied immunogen dose and the



outcome of vaccination. Various metered devices can be used for the purpose.

In one more preferred embodiment of the method according to the present invention a suspension of antigen-free penetrants is loaded with the antigen to be associated therewith during the day prior to an administration, preferably 360 min, more preferably 60 min and even more preferably 30 min before administering the resulting formulation in the nose.

In another preferred embodiment of the method according to the present invention at least one dose of vaccine is administered.

This embodiment of the method of the invention includes the repeated administration of the vaccine of the invention. Repeated administration includes repeated administration in the nose or one or more administrations in the nose in combination with conventional, e.g. parenteral administrations. In this connection, the kit of the invention may be advantageously made to comprise one or more containers, ampules or other kind of units comprising the vaccine of the invention.

In a particularly preferred embodiment of the method according to the present invention the time interval between the subsequent vaccinations is chosen to be between 2 weeks and 5 years, often between 1 month and up to 3 years, more frequently between 2 months and 1.5 years.

In a further preferred embodiment, repeated immunogen administration is advocated to maximise the final effect of a therapeutic vaccination. It is proposed to use between 2 and 10, often between 2 and 7, more typically up to 5 and most preferred up to 3 immunisations, when a non-allergenic antigen is used, or such a number of times, in the case of allergens, as is required either to achieve the desired immuno-tolerance, determined as described above or by some other suitable assessment method, or else to deem the effort as having failed. The time interval between subsequent vaccinations should preferably be between 2 weeks and 5 years, often between 1 month and up to 3 years, more frequently between 2 months and 1.5 years, when a subject is being immunised for the first time. Rodents, such as mice and rabbits are advantageously immunised in 2 weeks interval, primates, e.g. monkeys and often humans, need a booster vaccination in 3-6 months interval.

5 In another preferred embodiment of the method according to the present invention the flux of penetrants that carry an immunogen through the various pores in a well-defined barrier is determined as a function of the suitable driving force or a pressure acting  
10 across the barrier and the data are then conveniently described by a characteristic curve which, in turn, is employed to optimise the formulation or application further.

15 The disclosure content of the documents cited throughout this specification are herewith incorporated by reference. Further incorporated by reference is the complete disclosure content of the co-pending application filed in the name of IDEA AG and bearing the title "Noninvasive vaccination through the skin".

20 The figures show:

25 Figure 1 illustrates the effect of nasal insulin administration by means of carriers in an insulin dependent diabetes mellitus patient, with the result of an i.v. injection of fast-acting insulin (Actrapid, Novo-Nordisk) shown in the inset for reference.

30 Figure 2 illustrates the glucodynamics in a healthy human volunteer following intranasal administration of insulin by means of Transfersomes. Inset gives the result of intravenous injection of similar formulation for reference purpose.

35 Figures 3a and 3b provide further examples measured with a healthy volunteer following intranasal administration of insulin formulations with inferior characteristics, believed to be due to too slow drug release from the carrier.

40 Figure 4 illustrates the capability of nasally administered cytokines, associated with Transfersomes, to affect the outcome of transnasal immunisation with tetanus toxoid.

45 Figure 5 illustrates the biodistribution of insulin-derived radioactivity in mice following nasal administration of the agent in transfersomes.

50 Figure 6 gives the corresponding results for interferon, as measured in mice.

5 Figures 7 illustrate the effect of changing aggregate size and/or deformability on TT  
specific immune response in mice treated with various mixed micelles, Transfersomes  
or liposomes loaded with TT. Panels a and b show antibody isotype patterns, and in  
10 panel c the total antibody titre, as expressed in absorbency change is given.

15 Figures 8 highlight the (small) effect of changing antigen dose (in the high dosage  
range) on transnasal immunisation of mice with TT by means of Transfersomes with or  
without lipid A derivative as an immunoadjuvant. In panel a, the results of total  
absorbance measurements are given, panel b shows the corresponding titration  
curves, and panel c gives the relevant antibody isotypes.

20 Figure 9 is organised in similar fashion to compare the outcome of intranasal, oral or  
subcutaneous TT administration using different antigen doses and purity.

25 Figure 10: For comparison, animal protection (survival) data are given for the  
experiments in which several doses and administration routes were compared.

30 Figure 11 presents a set of data on the effect of various cytokines, or their  
combination, on the murine immune response to TT administered into the nose by  
means of transfersomes, with s.c. data given for comparison. Panel a gives the  
absorbance and titre data and panel b contains the isotype distribution results.

35 Figure 12 deals with the effects of combining low and high molecular weight immuno-  
adjuvants (lipid a analogue and interleukin-12).

40 Figure 13 illustrates the effect of specific cytokine inducers of microbial origin. Cholera  
toxin (CT) is used for the purpose.

45 Figure 14 shows one effect of heat labile toxin from E. coli as immuno-adjuvant.

50 Figure 15 illustrates the results obtained with a combination of two antigens, tetanus  
toxoid and cholera toxoid.

The examples illustrate the invention.

## EXAMPLES

### General experimental set-up and sample preparation

**Conventional vesicles, liposomes**, comprised soy phosphatidylcholine (SPC; Nattermann Phospholipids, Rhone-Poulenc Rorer, Cologne, Germany).

The suspension containing 10 w-% of the lipid in form of multilamellar vesicles was prepared by suspending the lipid in a buffer and then extruding the suspension through several polycarbonate membranes (with 800 nm, 400 nm, 200 nm and 100 nm pores, respectively) to narrow down the final vesicle size distribution. If required, as judged on the basis of optical inspection or the dynamic light scattering done after the latter steps, extrusions were repeated several (up to 5) times. In some cases, the vesicles were first extruded to a diameter of app. 50 nm and then frozen and thawed three times to enlarge the vesicles again, owing to inter-vesicle fusion. Subsequently, the formulation was passed through a micro-porous filter (100 nm; Poretics, CA), under pressure, to prepare the final suspension of oligo- or unilamellar vesicles.

**Highly adaptable penetrants**, used in the described examples, typically had the form of ultradeformable vesicles (Transfersomes™) with one or a few bilayers. They comprised a mixture of phosphatidylcholine and (bio)surfactants (cholate or polysorbate (Tween 80)), and various biologically active ingredients, such as insulin, interferon, interleukin, or GC-SF.

The above mentioned penetrants were prepared by mixing the phospholipid(s) with a suitable membrane-softening agent, such as cholate or polysorbate, as the case may be, either in an aqueous buffer or in ethanol; occasionally chloroform was used. In the latter two cases, which gave similar results, the solvent was evaporated under vacuum (10 Pa, overnight). The resulting lipid film was then hydrated with a buffer (pH around 7) to get a 10 wt-% lipid suspension, by and large. Vesicles were brought to the final, desired size by sequential extrusion as described for liposomes, using mainly filters with smaller pore sizes. The final size of Transfersomes was similar to that of liposomes.

5 Changing the surfactant-to-lipid ratio is believed to affect the mixed lipid bilayers deformability: the higher the surfactant concentration, the more adaptable is the  
10 resulting aggregate, up to the concentration at which the mixed lipid membranes became unstable, owing to the high surfactant concentration. At such point the mixed aggregates revert into micelles which no longer change their shape easily, owing to the  
15 low compressibility of the micelle interior. Vesicles without a surfactant or some other edge active ingredient, which are commonly known as liposomes and have at least 10x less flexible membranes than the more deformable mixed lipid vesicles, are a convenient negative control for the latter. The other obvious control are

20 **Mixed lipid micelles** containing similar ingredients as the corresponding highly adaptable penetrants, but in a different ratio, such that the edge active component (typically, but not necessarily, the surfactant) concentration is above the solubilization  
25 concentration value. To prepare said micelles, individual components were mixed in the aqueous phase and permitted them to interact until the mixture became optically clear, that is, solubilised, as judged by optical inspection or absorption measurement at 400 nm to 600 nm.

#### 30 Experiments carried out on human volunteers

35 To test biological activity of insulin carriers in humans, a freshly prepared test formulation was used in the nose of two test subjects. The first was a normoglycaemic (male, 74 kg, 173 cm, 45 years); the second was a C-peptide negative IDDM patient (female, 62 kg, 167 cm, 26 years). The test persons fasted between 6 h and 12 h prior  
40 to insulin administration.

45 To follow the temporal variation of glucose concentration in the blood, 5  $\mu$ L to 30  $\mu$  samples taken, every 10 min to 15 min, from the fingers on both arms. After an initial test period, during which the 'normal' blood glucose concentration and/or its change was determined, a suspension of carriers loaded with insulin (Transfersulin) was sprayed into each nostril, using conventional non-metered sprayer, in a series of 150  
50  $\mu$ L puffs. Care was taken to minimise the spill-over of test formulation into the throat or the dropping of said formulation from the nose.

Commercial glucometer (Accutrend™, Boehringer-Mannheim) was employed to determine the blood sugar concentration. At each time point, three individual, independent readings were made, except when the standard deviation was so high as to require repeated measurements.

The test formulations were made essentially as described in patent application PCT/EP98/06750. In brief, a suspension of highly adaptable penetrants with the above mentioned composition and an average diameter of the order of 100 nm to 150 nm was loaded with the drug, based on interfacial adsorption, and used within 24 h after the preparation. The drug-carrier association in the formulation was determined to be between 60% and 70%.

To administer the drug laden suspension into the nose, the preparation was filled into a commercial nebuliser (with a hand-driven pump, vertically oriented spraying nozzle and a puff volume of 150  $\mu$ L, on the average). One puff was given into each nostril at a time, while the test subject gently sniffed.

The total number of puffs was a function of the application dose (in this case: 2). Immediate spill-over into the throat or partial leakage of the fluid from the nose was reported in 10-20% of cases. No side effects, such as local irritation, sneezing, etc., were observed.

**Example 1:**

28.4 mg/mL phosphatidylcholine from soy-bean  
9.5 mg/mL phosphatidylglycerol from soy-bean  
62.1 mg/mL Tween 80  
phosphate buffer, pH 7.4  
human recombinant (hr) insulin, 50 IU/mL  
(from Actrapid 100 HM™, Novo-Nordisk)

Applied dose: ~5 IU per nostril

**Results** measured with a healthy subject are shown in figure 1. They reveal a transient decrease in the systemic blood glucose concentration after two administrations of the

5 drug in carriers (closed symbols), with a maximum after 20-30 min and a return to the pre-treatment value after approximately 1 h in either case. The observed change in glucose level corresponds to approximately 8.5% of the decrease was measured in an independent experiment after intravenous injection of the drug (Inset: open symbols).  
10 The reproducibility remains to be improved, however, the first application, biased by the lack in administration skill having been less successful than the second administration.

15 No irritation or other unpleasant sensation was reported by the test person after nasal administration of insulin in highly adaptable penetrants.

20 **Example 2:**

*Insulin loaded, highly adaptable carriers in an IDDM patient*

25 highly adaptable penetrants:  
as given in example 1

30 Applied dose: 25 IU per nostril

Test preparation and experiment was performed as described with previous example. The last administration of conventional insulin (Monotard™, Novo-Nordisk), at the dose  
35 of 22 IU was done at 10 p.m. on the previous day. Test subject, moreover, was stabilised by using long-acting insulin on the test day prior to nasal administration of the insulin associated with highly adaptable drug carriers.

40 **Results** of an experiment done with said IDDM patient is illustrated in figure 2. Owing to the lack of endogenic insulin production in this test subject, the pre-treatment blood glucose concentration was slightly above the normal, but relatively constant. The  
45 change resulting from nasal drug administration with ultra-adaptable carriers, has more a step-like rather than a peak-like shape (closed symbols), completed within 75 min. This is precisely what one would expect for an IDDM patient. The result of an i.v.  
50 injection of rapidly acting insulin (Actrapid™, Novo-Nordisk) in the same test person on a different occasion (inset: open symbols) corroborates the conclusion. An estimate of

5        apparent bioavailability of nasal insulin based on these data is around 4% and, consequently, somewhat lower than that reported in example 1. This may have to do with the presumed variability in drug release between different formulations which is illustrated in the following examples.

10        Nasal administration of carrier-associated insulin, according to the test person, caused no adverse side effect, locally or systemically.

15        **Examples 3-5:**

20        *Insulin associated with suboptimal carriers*

25        **Carriers**

as in previous examples, but believed not to release the drug readily owing to the higher affinity of selected insulin batch for the carrier, which makes the drug adsorption irreversible.

Applied doses: 50 IU, 50 IU

30        **Results** of the test measurements done with several different vesicle suspensions, illustrated in figure 3, signal lack of action for the insulin administered nasally with such carriers. The blood glucose concentration in the investigated normoglycaemic test person remains the same before, during and after the drug administration, for several hours at least. This suggests that the mere presence of carriers, or their ingredients, is insufficient to improve the bioavailability of nasally applied macromolecules, such as insulin. To achieve the desired biological effect, the rate of drug release from the carrier must also be adequate, such rate being determined in dedicated ex vivo experiments by using conventional protein binding deassociation techniques.



**Animal experiments****Examples 6-9 :***Labelled insulin delivery across nasal mucosa of test mice*

highly adaptable penetrants:

87.4 mg/mL phosphatidylcholine from soy bean (SPC)

12.6 mg/mL of a 50 % ionised cholic acid

phosphate buffer, 50 mM, pH 6.5

hr-Insulin (Actrapid™, Novo-Nordisk)

labelled insulin from Amersham

(345  $\mu$ L contain 1.08  $\mu$ g insulin and 1.725 mg BSA)

<sup>125</sup>I-labelled insulin (210  $\mu$ L) was mixed with 210  $\mu$ L of hr-insulin (Actrapid™ Novo-Nordisk, 100 HM) and purified 2 times by centrifugation to eliminate the non-bound label, which diffuses across the barrier much faster and better than whole drug molecules. 100  $\mu$ L of the resulting solution was mixed with 150  $\mu$ L phosphate buffer to yield pH around 7. Protein solution and lipids were processed together, bringing the final vesicle size by repeated extrusion through 100 nm pore filters to values around 150 nm.

Mice of NMRI strain (36 g to 51 g) from a local supplier were kept in suspension cages in groups of 4 to 6. The animals had free access to standard chow and water. Each mouse received 2.5  $\mu$ L of labelled penetrant suspension containing insulin per nostril. Then, the decrease in total radioactivity was assessed by whole-body camera at least 2 times. At different times the mice were killed and all major organs were taken and measured separately. The carcass was measured in two steps, after organ elimination and then after separation of the head. Radioactivity in excrement and cage was also determined.

**Results** pertaining to different time-points are given in Figure 4. They show that substantial amount of nasally administered radioactivity is recovered from the body, even after exclusion of gastro-intestinal tract, especially during the first hours following

suspension administration. Values in the blood are in the range of 9% at 0.5 h and 2%, the specific concentration falling from 3%/mL at the beginning to 0.7%/mL at the end. Activity in the nose decreases from 10.4% at 0.5 h to 0.3% at 8 h. Liver values are between 2.3% after 0.5 h, the maximum around 2.8 at 1 h and values above 1% after 4 h. After 8 h, the residuum in the liver is around 0.4%. The relatively high hepatic values are suggestive of passage of particles, that is, penetrants, through the barrier and subsequent uptake in the reticulo-endothelial system.

Corresponding CNS values are 0.1% and 0.03%. Maximum in the brain is measured between the first and second hour with app. 0.11% and 0.14%, respectively, which amounts to around 0.3%/g organ. These, apparently low values compare favourably with the result of more conventional drug delivery into CNS which yields values below 0.5% of injected dose or around 0.15%/g organ, for example, when transferrin-receptor is used to deliver the drug (Pasechnik & Price, 1996). In the case of white-germ agglutinin 0.1% was found in olfactory bulb.

#### Examples 10-11 :

highly adaptable penetrants

87.4 mg/mL phosphatidylcholine from soy bean (SPC)

12.6 mg/mL of a 50% ionised cholic acid

phosphate buffer, 50 mM, pH 6.5

human recombinant insulin (Actrapid™, Novo-Nordisk)

labelled insulin from Amersham

In a related experiment, 345  $\mu$ L of  $^{125}$ I-labelled insulin was mixed with 345  $\mu$ L of cold Actrapid™ (Novo-Nordisk) and purified 2 times, as in previous experiment. After addition of 200  $\mu$ L phosphate buffer, 150  $\mu$ L of resulting solution was mixed with the lipids and extruded to final vesicle size. The applied dose was 3  $\mu$ L per nostril. Mice were killed after 1 h, fixed, cut in thin sections and inspected by the whole-body radiography. Free insulin in solution was used for comparison.

The results of above mentioned experiments (not shown) revealed high label accumulation in the nasal region, as one would expect, substantial spill-over into the GI

tract, very high density in the bladder, but also some radioactivity in the liver, which appears to be slightly higher for the carrier-derived than for the free insulin.

#### Examples 12-13:

##### *Labelled interferon-gamma delivery across nasal mucosa of test mice*

highly adaptable penetrants

86.6 mg/mL phosphatidylcholine from soy bean (SPC)

13.4 mg/mL Na cholate

phosphate buffer, 10 mM, pH 7.2 (nominal)

1 mg IFN-gamma/mL suspension

(100  $\mu$ Ci/mL suspension, as 3-<sup>125</sup>I-tyrosyl-IFN-gamma)

Applied dose: 5  $\mu$ L nostril

Mice of NMRI strain (36 $\pm$ 0.6 g) were housed and taken care of as described with previous examples. Prior to the test formulation application, the animals were sedated as described before. Test formulation then was administered through a fine catheter in two drops of 5  $\mu$ L, resulting in the total dose of 1 mg lipid. After this, the animals were kept in separate cages to prevent mutual contamination.

Measured radioactivity in the blood was found to correspond to app. 2.5% of the applied dose, liver concentration being at app. 2% and colon concentration around 2.5%, all after 2 h. The highest amount of radioactivity by then was recovered from the stomach (37%) and in the cage plus excrement (32%).

In the central nervous system (CNS) 0.06% of total nasally applied dose, as judged by derived radioactivity, was present 1 h after the drug administration by means of highly adaptable, protein-loaded mixed lipid-surfactant vesicles.

#### Examples 14-19:

##### *Cytokine delivery across the nasal mucosa of test mice*

highly adaptable penetrants

37.7 mg/mL phosphatidylcholine from soy bean (SPC)

62.3 mg/mL polysorbate (Tween 80)

phosphate buffer, 10 mM, pH 6.5

Tetanus toxoid, as antigen (2 mg/mL)

Interferon- $\gamma$  (IFG- $\gamma$ )

Granulocyte-monocyte-colony stimulating factor (GM-CSF)

Interleukin 4 (IL-4)

Interleukin 12 (IL-12)

Applied dose: 3  $\mu$ L per nostril

Mice of Swiss albino strain (18-20 g) were obtained from The National Institute of Nutrition (Hyderabad, India). They were 8 to 12 weeks old at the time of first immunisation. The antigen alone or in combination with various cytokines, both believed to be at least partly associated with the carriers, was positioned with a sequencing in front of the animal nose and left to be sucked-in by the latter. Blood samples were collected retro-orbitally and tested with specific antibodies directed against the employed antigen by measuring absorbance at 492 nm, after subtraction of blank samples with ELISA.

The results of above mentioned measurements, illustrated in figure 6, suggest that the presence of all tested cytokines in vaccination formulation, based on the highly adaptable antigen carriers, increases the serum absorbance compared to that characterising the non-modulated value, determined after simple immuno-carrier administration. Relative differences are more likely consequences of diverse bio-potency of tested immuno-modulants employed in the present specific experimental system than indicative of variable macromolecular transport rate across the nasal mucosa.

The observed 100% increase in serum absorbance measured for GM-CSF/IL-4 combination is remarkable, as it is known that neither polysorbate nor phosphatidylcholine ex soy-bean can markedly enhance permeation capability on their own. It is therefore reasonable to assume that the observed effect is not simply due to the delivery of antigen molecules (with the molar mass of 150 kDa) across the nasal

5 mucosa but, moreover, testify that at least a proportion of co-administered cytokines has passed the barrier in a biologically active form.

**Examples 20-21:**

10 highly adaptable penetrants

as in examples 14-19, except for the absence of cytokines

15 Tetanus toxoid antigen (2 mg/mL)

Mixed lipid micelles

14.8 mg/mL phosphatidylcholine from soy bean (SPC)

20 85.2 mg/mL polysorbate (Tween 80)

phosphate buffer, 10 mM, pH 6.5

Tetanus toxoid antigen (2 mg/mL)

25 Applied dose: 3  $\mu$ L per nostril

Experiments were done as described with previous examples (14-19).

30 Immune response in the animals treated with mixed lipid micelles as in Examples 14-19 was clearly inferior to that measured after the nasal application of antigen in the highly adaptable lipid vesicles, despite the fact that the latter contained a smaller  
35 amount of Tween 80 than the former. If the surfactant was responsible for the transport of macromolecules across nasal mucosa, owing to its action as skin permeation enhancer, precisely the opposite experimental outcome would have been expected.

40 This suggests that highly adaptable carriers (mixed lipid vesicles) transport macromolecules across the nasal mucosa by a mechanism other than the drug permeation.  
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**Examples 22-29:**Aggregate size (stability) effect

Highly deformable vesicles with NaCh (Transfersomes™)

89.3 mg phosphatidylcholine from soy bean

10.7 mg sodium cholate (NaCh)

0.9 mL phosphate buffer, pH 6.5

(Mixed lipid) Micelles with NaCh, type 1

65 mg phosphatidylcholine from soy bean

35 mg sodium cholate

0.9 mL phosphate buffer, pH 6.5

(Mixed lipid) Micelles with NaCh, type 2

31.6 mg phosphatidylcholine from soy bean

68.5 mg sodium cholate

0.9 mL phosphate buffer, pH 6.5

Highly deformable vesicles with Tw, Transfersomes™ type 1

37.7 mg phosphatidylcholine from soy bean

62.3 mg Tween 80 (Tw)

0.9 mL phosphate buffer, pH 6.5

Highly deformable vesicles with Tw, Transfersomes™, type 2

64.5 mg phosphatidylcholine from soy bean

35.5 mg Tween 80

0.9 mL phosphate buffer, pH 6.5

(Mixed lipid) Micelles with Tw, type 1

13.2 mg phosphatidylcholine from soy bean

86.8 mg Tween 80

0.9 mL phosphate buffer, pH 6.5

(Mixed lipid) Micelles with Tw, type 2

7 mg phosphatidylcholine from soy bean

93 mg Tween 80

0.9 mL phosphate buffer, pH 6.5

0.10

Lipid vesicles (liposomes)

65 mg phosphatidylcholine from soy bean (SPC)  
35 mg cholesterol  
0.9 mL phosphate buffer, pH 6.5

Tetanus toxoid (2 mg/mL; home made) used at the dose of  
40  $\mu$ g (20  $\mu$ L) TT per mouse and immunisation

The medium filtrate from a culture of *Clostridium tetani* grown in vitro was used as an  
purified antigen. Pure toxoid was purchased from Accurate Antibodies, NY, USA.

To test the effect of aggregate properties in the formulation, three kind of aggregates  
were prepared: relatively large vesicles (diameter between 100 nm and 200 nm) either  
comprising a flexible membrane (Transfersomes) or a relatively rigid membrane  
(liposomes) and much smaller micelles (diameter below 50 nm). The latter were  
chosen to mimick the more conventional approach of using detergents as nasal  
mucosa permeation enhancers.

Amongst the eight tested formulations, Transfersomes, on the average, give best  
results, but absolute titres are always very low, probably owing to the antigen impurity.  
Mixed lipid micelles are most efficient in creating IgA, but are not really different than  
the other aggregates in the case of IgG2a and IgM, whilst in the case of IgG2b they are  
comparable to Transfersomes. The IgG1 level, which is decisive for animal protection,  
is only significantly elevated when Transfersomes are used to deliver TT across  
through the nose (see figure 7a).

Mixed micelles containing less potent detergents (with lesser skin permeation  
enhancing capability) are, relatively speaking, less efficient 'immuno-carriers' (see  
figure 7b), the more deformable Transfersomes with a higher Tw content standing  
clearly out in the case of IgG2a and IgM, are similar to the less deformable  
Transfersomes with a lower Tw content in the case IgG1 and IgG3, and are as efficient  
as mixed micelles with Tw in the case of IgA and IgG2b. The smallness of measured  
values is reason for the concern, however, which can best be overcome by using purer  
antigen.

Looking at the cumulative titre of all specific anti-TT antibodies in the serum, liposomes are relatively efficient 'immuno-carriers' in the primary and mature response (perhaps owing to the action of non-associated TT), whilst the Tw rich mixed micelles are the worst. NaCh Transfersomes are top performers in the late immune response (cf. figure 7c).

#### Examples 30-35:

##### Antigen dose and purity effect

##### Highly deformable vesicles (Transfersomes):

86.3 mg phosphatidylcholine from soy bean (SPC)  
13.7 mg sodium cholate (NaChol)  
+/- 0.04 mol-% monophosphoryl Lipid A (LA) relative to SPC  
0.9 mL phosphate buffer, 10 mM, pH 6.5

Tetanus toxoid (TT, from local source, purified by ultrafiltration)

0 µg, 40 µg or 80 µg TT/ mouse/ immunisation

To obtain partially purified antigen, such filtrate was passed through a 10 kDa cut-off membrane and washed thoroughly with phosphate buffer, pH 6.5; in the process, the culture filtrate was concentrated 15 times.

Dose dependence results are illustrated in figure 8a. The TT-specific increase in serum absorbance following TT administration through the nose by means of Transfersomes reveals a positive dose dependence in the primary and late immune response in the absence of LA, the presence of LA reverting this trend. Titre-wise and with regard to specific antibody isotype distribution, similar but not identical picture is obtained (cf. figures 8b and 8c). The survival data are indicative of good protection in every case. Taken together this suggests that the required dose for non-invasive nasal immunisation by means of highly deformable carriers is much lower than that required for a successful non-invasive TT administration through the skin.



Antigen purity effect. Comparison of the data shown in figure 8c and 7a and 7b shows that antigen purity strongly affects the level of murine immune response against tetanus toxin when the toxoid has been applied non-invasively through.

#### Examples 36-46 :

##### Route of administration

Highly deformable vesicles, NaCh Transfersomes™

as described with examples 1-8

Tetanus toxoid mixed with NaCh suspension

20 mg/mL sodium cholate in  
phosphate buffer, pH 7

Tetanus toxoid dose: 40 µg TT per immunisation; 5 µg TT, 10 µg TT, 20 µg TT, 40 µg TT per immunisation.

Using the same experimental procedures as described with previous examples, the antibody-specific serum absorbance the corresponding antibody titre and isotype distribution, and the level of animal protection against tetanus toxin was determined after nasal, oral and subcutaneous antigen administration.

The results are given in figures 9. They reveal that the increase in serum absorbance, ultimately, is comparable after invasive and non-invasive antigen administration (figure 9a). However, the titre in the latter case is significantly lower except in the primary response. Interestingly, s.c. injection only produces superior results after the second boost, whereas the combination with TT and cholate, which then can act as nasal permeation enhancer in total antibody titre is better at all times. The probable reason for this is the high concentration of IgG2b, as is seen from figure 9b. Injections elicit most efficiently the IgG1 and IgM type of antibodies.

Animals are well protected by any of above mentioned vaccinations with TT, but only after 2 boosts; in the case of nasal vaccination. In contrast, one boost is sufficient

(data not shown). Using 4-8x lower doses of purified TT suffices for protection in the case of nasal vaccination, but not when the antigen is injected (cf. figure 10).

#### Example 47

##### Low molecular weight adjuvant (lipid A) effect

Highly deformable immuno-modulated TT-Transfersomes™:  
as in examples 9-14

Tetanus toxoid: 2 mg/mL, with 20  $\mu$ L or 40  $\mu$ L corresponding to 40  $\mu$ g or 80  $\mu$ g TT per immunisation

It is believed that co-administration of immuno-active, typically immunopotentiating, molecules is advantageous for presentation TT associated with the carriers to the body. To substantiate this conclusion specifically the outcome of non-invasive immunopresentation of TT was compared by means of Transfersomes with or without monophosphoryl lipid A (LA), which is a well known immunostimulant known to elicit generation of TNF, for example. For the used, relatively high antigen doses no strong dependence was found, however. In either case substantial titres and a prophylactic immune response was reached, which was not the case with purified TT which profited from the presence of LA.

#### Examples 48-53:

##### High molecular weight immunomodulators (cytokines)

Highly deformable vesicles, Tw Transfersomes™:

as described with examples 1-8, plus

various cytokines (Interferon- $\gamma$ , GM-CSF, IL-4, IL-12)

(0.05 mg IFN- $\gamma$ ; 0.004 mg GM-CSF; 0.004 mg IL-4 per mL, 0.004 mg IL-12 per mL)

Tetanus toxoid, 2 mg/mL, corresponding to 40  $\mu$ g TT (purified, home prepared) per mouse/ immunisation

5 The effect of cytokines was studied individually and in combination. The results are  
given in figures 5. They suggest that GM-CSF plus IL-4 combination can support the  
10 generation of anti-TT antibodies in mice, as can, probably, IFN- $\gamma$  and perhaps IL-12,  
and maybe IL-4 (cf. figure 11a). The strongest effect is seen in the case of IgM and  
IgA, except in the case of IL-12 usage, which only affects strongly IgG2b generation.  
15 The protection relevant IgG1 is increased strongly only by the combination of GM-CSF  
and IL-4, whereas IgG3 is not affected at all. Injection works best for IgG1 (cf. figure  
11b).

#### 20 Examples 54-58:

##### Combination of low and high molecular weight adjuvants (LA + IL-12) effect

25 Highly deformable vesicles, NaCh Transfersomes™,  
as described with examples 1-8, plus  
0.4 mg IL-12 per mL immunogen suspension  
0.04 mol-% monophosphoryl Lipid A (LA) relative to SPC

30 Tetanus toxoid (purified), 2 mg/mL, corresponding to 40  $\mu$ g TT per mouse/  
immunisation

35 The effect discussed with examples 25-31 was confirmed for a blend low molecular  
and high molecular weight immunoadjuvants. The results are given in figures 12 and  
show that the immunopotential by such a combination is especially strong during  
40 the early stage of immune response, the combination in any case being better than LA  
alone.

#### 45 Examples 59-71:

##### Bacterial wall component, cholera toxin, as adjuvant:

50 Highly deformable vesicles, Transfersomes™ (Tfs):  
TfsC

86.3 mg phosphatidylcholine from soy bean (SPC)

13.7 mg sodium cholate (NaChol)

0.9 mL phosphate buffer, 10 mM, pH 6.5

0.1 mL ethanol

TfsT

36 mg phosphatidylcholine from soy bean (SPC)

64 mg Tween 80

0.9 mL phosphate buffer, 10 mM, pH 7

Cholera toxin (CT; Sigma, Neu-Ulm), 2  $\mu$ g/immunisation, if specified,

Tetanus toxoid (TT, pure; Accurate Antibodies), 2 mg/mL.

Volume doses corresponding to 0  $\mu$ g TT/mouse/immunisation (negative control), 1  $\mu$ g TT/mouse, 5  $\mu$ g TT/ mouse, 10  $\mu$ g TT/ mouse, 20  $\mu$ g TT/mouse, 40  $\mu$ g TT/mouse (in the absence of CT) and 0.5  $\mu$ g TT/mouse/immunisation, 1  $\mu$ g TT/mouse, 5  $\mu$ g TT/ mouse (when using CT) was used intranasally in the type T Transfersomes (TfsT) in both nostrils and at the dose of 0.5  $\mu$ g TT/mouse/immunisation in the type C Transfersomes (TfsC) in 4-6 Swiss albino mice. Moreover, 20  $\mu$ g TT/ mouse/immunisation in TfsT was injected subcutaneously at the corresponding site in the positive control group. Immunisations were done on days 1, 14, 28.

The protective effect of antigen applied in the nose was good when the antigen dose exceeded 20  $\mu$ g/immunisation; lower doses yielded insufficient, but detectable protection (cf. figure 13). When cholera toxin (CT) was included into the test formulation together with the tetanus toxoid, excellent protection was achieved already at the lowest of tested doses (0.5  $\mu$ g/immunisation), independent of the ultra-deformable carrier composition. Protection was complete in all test groups containing CT in the formulation applied on the skin.

#### Examples 72-74:

Heat labile toxin from E. coli (HLT) as immuno-adjuvant

Highly deformable vesicles, Transfersomes™:

TfsC

86.3 mg phosphatidylcholine from soy bean (SPC)  
13.7 mg sodium cholate (NaChol)  
0.9 mL phosphate buffer, 10 mM, pH 6.5  
Heat Labile Toxin (HLT, SIGMA, Neu-Ulm),  $\leq 1$  mg/mL plus, if required  
Tetanus Toxoid (TT, pure, Accurate Antibodies) 2 mg/mL

or

TfsT

36 mg phosphatidylcholine from soy bean (SPC)  
64 mg Tween 80  
0.9 mL phosphate buffer, 10 mM, pH7  
Heat Labile Toxin (LT, SIGMA, Neu-Ulm),  $\leq 1$  mg/mL, if required,  
Tetanus Toxoid (TT, pure, Accurate Antibodies) 2 mg/mL

Antigen carriers were prepared and all experiments (with Swiss albino mice) and assays were carried out as described in the previous examples. A series of different HLT doses ranging from approx. 50 ng/application to multi-microgram quantity per application was used in combination with TT concentrations in the range of approx. 100 ng and up to 10  $\mu$ g per application. In most cases, volume doses corresponding to 0.5  $\mu$ g TT / mouse / i.n. immunisation and 0.1 to 0.5  $\mu$ g HLT / mouse / i.n. immunisation and a positive control with 0.5  $\mu$ g TT for s.c. injection was used for mice immunisation.

As is shown in Figure 14, anti-TT titres are improved by HLT acting as an adjuvant in comparison with the result of s.c. injection without an adjuvant. The humoral response is dose dependent, i.e. higher anti-TT titres are achieved with the higher dose of HLT. The correlation is not linear, however, but shows rather a maximum (data not shown).

Protection against tetanus toxin challenge is equally efficient for the high and the low dose of immuno adjuvant.

This suggests that adjuvants should be used in the nose in conjunction with Transfersomes at maximum at the dose that is in the low-end range of doses used for conventional invasive (s.c.) immunisations; the minimum dose for immuno-Transfersomes usage in the nose should also be 1-2 orders lower, for the adjuvant tested in this series for most, if not all, immunoadjuvants.

**Example 75:**Bivalent vaccination with Tetanus Toxoid and Cholera Toxin as antigens

Highly deformable vesicles, Transfersomes™:

86.3 mg phosphatidylcholine from soy bean (SPC)

13.7 mg sodium cholate (NaChol)

0.9 mL phosphate buffer, 10 mM, pH 6.5

Cholera Toxin (CT, SIGMA, Neu-Ulm),  $\leq 1$  mg/mL

Tetanus Toxoid (TT, pure, accurate antibodies) 2 mg/mL

or

TfsT

36 mg phosphatidylcholine from soy bean (SPC)

64 mg Tween 80

0.9 mL phosphate buffer, 10 mM, pH7

Cholera Toxin (CT, SIGMA, Neu-Ulm),  $\leq 1$  mg/mL

Tetanus Toxoid (TT, pure, accurate antibodies) 2 mg/mL

Details on the formulation and its preparation, on vesicle characterisation and on animal experiments as well as the following assays are given in the description of related examples hereinabove.

The main result of this experimental series was that Cholera Toxin, added to the formulation as an adjuvant, can also induce the formation of anti-CT antibodies in practically relevant quantity. This effect can, but does not have to, be achieved using concentrations of CT and TT sufficient to ensure good protection against the challenge with tetanus toxin. Figure 15 compares anti-TT and anti-CT titres from the same mice immunised with TT and CT within the same carrier. This reveals the potential of Transfersomes™ containing more than one antigen to serve as the basis for at least bivalent vaccines.

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## Claims

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CLAIMS

1. Use of a penetrant, suspended or dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-like coating of one or several layers of at least two different substances or two different forms of a substance with the tendency to aggregate, said substances or forms of a substance differing by at least the factor of 10 in the solubility in a preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or form of the substance or the average diameter of the hetero-aggregates consisting of both said substances or forms of said substance is smaller than the average diameter of homo-aggregates of the less soluble substance or form of the substance and/or wherein the more soluble component tends to solubilise the penetrating droplet and wherein the content of such component amounts to up to 99 mol-% of the concentration required to solubilise the droplet or else corresponds to up to 99 mol-% of the saturating concentration in the un-solubilised droplet, whichever is higher, and/or wherein the elastic deformation energy of the droplet surrounding the membrane-like coating is at least 5x lower, more preferably is at least 10x lower and ideally is more than 10x lower than that of the red blood cells or of the phospholipid bilayers with fluid aliphatic chains, such droplets then acting as carriers for the transnasal administration of a pharmaceutically active compound, an antigen, an allergen, a mixture of antigens and/or a mixture of allergens.
2. Use of a penetrant, suspended or dispersed in a solvent, in the form of a minute fluid droplet surrounded by a membrane-like coating of one or several layers of at least two different substances or two different forms of a substance with the tendency to aggregate, said substances or forms of a substance differing by at least the factor of 10 in the solubility in a preferably aqueous, liquid medium, such that the average diameter of homo-aggregates of the more soluble substance or form of the substance or the average diameter of the hetero-aggregates consisting of both said substances or forms of said substance is smaller than the average diameter of homo-aggregates of the less soluble substance or forms of the substance and/or wherein the more soluble component tends to solubilise the penetrating droplet and wherein the content of such component amounts to up to 99 mol-% of the concentration required to

5 solubilise the droplet or else corresponds to up to 99 mol-% of the saturating  
concentration in the un-solubilised droplet, whichever is higher, and/or wherein  
the elastic deformation energy of the droplet surrounding the membrane-like  
coating is at least 5x lower, more preferably is at least 10x lower and ideally is  
10 more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains as a carrier for the preparation of a  
pharmaceutical, preferably a vaccine composition for transnasal administration.

- 15 3. Use of a penetrant, suspended or dispersed in a solvent, in the form of a minute  
fluid droplet surrounded by a membrane-like coating of one or several layers of  
at least two different substances or two different forms of a substance with the  
20 tendency to aggregate, said substances or forms of a substance differing by at  
least the factor of 10 in the solubility in a preferably aqueous, liquid medium,  
such that the average diameter of homo-aggregates of the more soluble  
substance or form of the substance or the average diameter of the hetero-  
25 aggregates consisting of both said substances or forms of said substance is  
smaller than the average diameter of homo-aggregates of the less soluble  
substance or form of the substance and/or wherein the more soluble component  
tends to solubilise the penetrating droplet and wherein the content of such  
30 component amounts to up to 99 mol-% of the concentration required to  
solubilise the droplet or else corresponds to up to 99 mol-% of the saturating  
concentration in the un-solubilised droplet, whichever is higher, and/or wherein  
the elastic deformation energy of the droplet surrounding the membrane-like  
35 coating is at least 5x lower, more preferably is at least 10x lower and ideally is  
more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains, said penetrant being used in combination with  
a pharmaceutically active ingredient or an allergen or an antigen for the  
40 preparation of a transnasally administerable pharmaceutical composition for the  
treatment of infective diseases, endocrine disorders, preferably hypopituitarism,  
diabetes, hyperthyroidism, thyroiditis, most preferably Hashimoto's thyroiditis,  
subacute thyroiditis; adrenal disorders, preferably Addison's disease, secondary  
adrenal insufficiency, Cushing's syndrome; gastrointestinal disorders, preferably  
45 Crohn's disease, colitis; hemorrhagic diseases, preferably hemophilia,  
leukopenia, hypereosinophilic syndrome; musculoskeletal and connective tissue  
disorders, preferably rheumatoid arthritis, Sjögren's syndrome, Bechet's

5 syndrome, lupus, scleroderma, polymyositis/dermatomyositis, polymyalgia  
rheumatica and temporal arthritis, polyarteriosis nodosa, Wegener's  
10 granulomatosis, mixed connective tissue disorder, ankylosing spondylitis,  
psoriatic arthritis, osteoarthritis, Paget's disease, sciatica, bursitis, tendonitis and  
tenosynovitis, epicondylitis, fibromyalgia, eosinophilic fasciitis; neurological  
disorders, preferably pain, singultus, vertigo, seizure disorders, sleep disorders,  
15 transient ischemic attacks, spinal cord injury, demyelinating diseases, nerve root  
disorders, myasthenia gravis; oncological disorders; psychiatric disorders,  
preferably drug dependence, neuroses, mood disorders, schizophrenic  
disorders, delusional disorders; and/or for use in the field of gynecology,  
20 preferably for the treatment of dysmenorrhea, menopause, chronic anovulation,  
premature ovarian failure, endometriosis, infertility; and/or for use in the field of  
immunology, preferably transplant rejection, hyposensitisation, allergen  
immunotherapy or prophylactic vaccination.

- 25 4. A pharmaceutical composition for transnasal administration comprising a carrier,  
which is a penetrant, suspended or dispersed in an aqueous solvent, in the form  
of a minute fluid droplet surrounded by a membrane-like coating of one or  
30 several layers of at least two different substances or two different forms of a  
substance with the tendency to aggregate, said substances or forms of a  
substance differing by at least the factor of 10 in solubility in a preferably  
aqueous, liquid medium, such that the average diameter of homo-aggregates of  
35 the more soluble substance or form of the substance or the average diameter of  
the hetero-aggregates consisting of both said substances or forms of said  
substance is smaller than the average diameter of homo-aggregates of the less  
soluble substance or form of the substance and/or wherein the more soluble  
40 component tends to solubilise the penetrating droplet and wherein the content of  
such component amounts to up to 99 mol-% of the concentration required to  
solubilise the droplet or else corresponds to up to 99 mol-% of the saturating  
45 concentration in the un-solubilised droplet, whichever is higher, and/or wherein  
the elastic deformation energy of the droplet surrounding the membrane-like  
coating is at least 5x lower, more preferably is at least 10x lower and ideally is  
50 more than 10x lower than that of the red blood cells or of the phospholipid  
bilayers with fluid aliphatic chains said composition also including a

5 pharmaceutically active ingredient, an allergen, an antigen, a mixture of antigens and/or a mixture of allergens.

- 10 5. The use of claim 3 or the pharmaceutical composition of claim 4 wherein the pharmaceutically active ingredient is an adrenocorticostaticum, an adrenolyticum, an androgen or antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum or analgesicum, an analepticum, an antiallergicum, antiarrhythmicum, antiarteroscleroticum, antiasthmaticum and/or
- 15 bronchospasmolyticum, an antibioticum, an anti-infective agent, antidepressivum and/or antipsychoticum, an antidiabeticum, an antidot, an antiemeticum, antiepilepticum, antifibrinolyticum, anticonvulsivum or anticholinergicum, an enzyme, a coenzyme or the corresponding enzyme inhibitor, an antihistaminicum (and combinations thereof) or antihypertonicum, an antihypotonicum, anticoagulant, antimycoticum, antimyasthenicum, an agent against Morbus Alzheimer or Morbus Parkinson, an agent for ACS therapy, an
- 20 antiphlogisticum, antipyreticum, antirheumaticum, antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, cardi tonicum, chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an anti-flew agent, a haemostaticum, hypnoticum, an immunoglobuline or its fragment or any other immunologically active substance, such as an immunomodulator, a bioactive carbohydrate (derivative), a contraceptive, an anti-migraine agent, a corticosteroid, a muscle
- 25 relaxant, a narcoticum, a neurotherapeuticum, a (poly)nucleotide, a neurolepticum, a neurotransmitter, a (poly)peptide (derivative), an opiate, an opthalmicum, (para)-sympaticomimeticum or (para)sympathicolyticum, a protein(derivative), a psoriasis/neurodermitis drug, a mydriaticum, a psychostimulant, rhinologicum, a sleep-inducing agent, a sedating agent, a spasmolyticum, tuberculostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, an alcohol abuse preparation, an anticonvulsant, an antineoplastic, an antirheumatic, an appetite suppressant, a biological response modifier, a blood modifier, a bone metabolism regulator, a cardioprotective agent, a cardiovascular agent, a central
- 30 nervous system stimulant, an enzyme, an agent for erectile dysfunction therapy, a fertility agent, a gastrointestinal agent, a gout preparation, a hormone, an agent for hypercalcemia management, an agent for hypocalcemia management,



an immunosuppressive, a migraine preparation, a motion sickness product, an agent for multiple sclerosis management, a muscle relaxant, a nutritional, an ophthalmic preparation, an osteoporosis preparation, an otic preparation, a parasympatholytic, a parasympathomimetic, a prostaglandin, a psychotherapeutic agent, a respiratory agent, a sedative & hyponotic, a skin & mucous membrane agent, a smoking cessation aid, a sympatholytic, a tremor preparation, a urinary tract agent, a vaginal preparation, a vertigo agent, an immunologically active substance (such as an immunomodulator, e.g., bacterial extracts or cell wall components like cholera toxin, heat labile toxin, monophosphorylipid A, or cytokine inducing agents or hormones like thymosin, thymulin, thymopoietin, or phytoimmunostimulants like extracts from Echinacea root, wild indigo root, white cedar leave tips, or synthetic immunomodulators like quinoline derivatives, synthetic peptides, pyrimidine, lipopeptides, or cytokines or immunosuppressants, and signal transduction inhibitors like cyclosporin A, FK506, FTY720, rapamycin), an inhibitor (antagonist), or a promotor (agonist) of the activity of any of above mentioned agents, or any combination of said active substances.

6. The use of claim 3 or the pharmaceutical composition of claim 4 wherein the antigen is derived from a pathogen.
7. The use of claim 3 or the pharmaceutical composition of claim 4 wherein said pathogen belongs to extracellular bacteria, including pus-forming cocci, such as *Staphylococcus* and *Streptococcus*, gram-negative bacteria, such as *Meningococcus* and *Gonococcus* species, species of *Neisseria*, gram negative bacteria, including enteric organisms such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Diphtheria*, *Bordetella Pertussis*, and gram-positive bacteria (e.g. *Bacillus pestis*, *BCG*), particularly anaerobes, such as the *Clostridium* species (e.g. *Clostridium tetani*, *Clostridium perfringens*, *Clostridium novyi*, *Clostridium septicum*), bacteria and viruses, which survive and replicate within host cells, comprising mycobacteria (e.g. *M. tuberculosis*) and *Listeria monocytogenes*, retro- and adenoviruses, including hepatitis virus, (human) immunodeficiency virus, herpes viruses, small-pox (chicken-pox), influenza, measles, mumps and polio viruses, cytomegalovirus, rhinovirus, etc., and fungi prospering inside host cells, a parasite including animal parasites, such as protozoa and helminths,

and ectoparasites, such as ticks and mites, or *Brucella* species, (e.g. *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. neotomae*, *B. ovis*, the causative agent for cholera (e.g. *Vibrio cholerae*), *Haemophilus* species like *H. actinomycentemcomitans*, *H. pleuropneumoniae*, as well as pathogens triggering paratyphoid, plague, rabies, tetanus and rubella diseases or to eukaryotic cells or their parts that cause various neoplasiae, auto-immune diseases and other pathological states of the animal or human body which do not necessarily result from microbial infections.

8. The use of claim 3 or the pharmaceutical composition of claim 4 wherein the antigen is used in a purified or even better in a pure form.
9. The use of claim 3 or the pharmaceutical composition of claim 4 wherein the antigen is the antigenic determinant of hepatitis virus, (human) immunodeficiency virus, herpes viruses, small-pox (chicken-pox), influenza, measles, mumps and polio viruses, cytomegalovirus, rhinovirus, etc., and fungi prospering inside host cells, a parasite including animal parasites, such as protozoa and helminths, and ectoparasites, such as ticks and mites, or *Brucella* species, including the causative agent for cholera, *Haemophilus* species, as well as pathogens triggering paratyphoid, plague, rabies, tetanus and rubella diseases or else eukaryotic cells or their parts that cause various neoplasiae, auto-immune diseases and other pathological states of the animal or human body, which do not necessarily result from microbial infections.
10. The use of claim 3 or the pharmaceutical composition of claim 4, wherein the allergen is of xenogenic or endogenic origin, derived from a microorganism, an animal or a plant, or belonging to the group of man made and/or irritating inorganic substances, or to such parts or components of the human body which were incorrectly processed by or exposed to the body immune system.
11. The use of claim 3 or the pharmaceutical composition of claim 4 wherein the allergen belongs to the class of the inhalation allergens, including but not limited to various pollen, spores, bits of animal hair, skin, feather, natural and synthetic textiles, wheat, (house) dust, including mite; furthermore, food and drug allergens; contact allergens; injection, invasion or depot allergens, such as

5 various (gastrointestine-resident) worms, echinococci, trichines, etc., a part of  
implantation material.

10 12. The use of any one of claims 1 to 3 and 5 to 11 or the pharmaceutical  
composition of any one of claims 4 to 9 additionally comprising a compound  
which releases or induces cytokine or anti-cytokine activity or exerts such an  
activity itself.

15 13. The use or the pharmaceutical composition of claim 12 wherein the compound  
exerting cytokine activity is IL-4, IL-3, IL-2, TGF, IL-6, TNF, IL-1 $\alpha$  and/or IL-1 $\beta$ , a  
type I interferon, preferably IFN-alpha or IFN- $\beta$ , IL-12, IFN- $\gamma$ , TNF- $\beta$ , IL-5 or IL-  
20 10.

25 14. The use or the pharmaceutical composition of claim 12 wherein said compound  
with anti-cytokine activity is an anti-cytokine antibody or the corresponding  
active fragment, a derivative, or an analogue thereof.

30 15. The use or the pharmaceutical composition of claim 4 wherein the compound  
displaying or inducing cytokine or anti-cytokine activity and the pharmaceutically  
active ingredient or antigen or allergen are associated with the penetrant.

35 16. The use of any one of claims 1 to 15 or the pharmaceutical composition of any  
one of claims 4 to 15 wherein the less soluble self-aggregating molecule is a  
lipid, preferably a polar lipid, and the more soluble component is a surfactant or  
some more soluble form of the polar/basic lipid.

40 17. The use of any one of claims 1 to 16 or the pharmaceutical composition of any  
one of claims 4 to 16 wherein the more soluble component is an agent to be  
transported across the barrier, said agent having a tendency to form common  
45 large structures with the less soluble component(s) of the penetrant, typically in  
the form of a physical or a chemical complex.

50 18. The use of any one of claims 1 to 17 or the pharmaceutical composition of any  
one of claims 4 to 17 wherein the more soluble component tends to solubilise  
the penetrating droplet and is present in concentration not exceeding 99 mol%

of the concentration required to disintegrate the droplet or, alternatively, not exceeding 99 mol% of the saturating concentration in the unsolubilised droplet, whichever is higher, values below 50% of the former relative concentration being particularly useful, with values below 40 rel-% or even around and below 30 rel-% being even more advantageous, whereas in the case of droplets which cannot be solubilised by the more soluble component relative concentrations which exceed the above mentioned relative concentrations by the factor of up to 2 are most preferred.

19. The use of any one of claims 1 to 18 or the pharmaceutical composition of any one of claims 4 to 18 wherein the less soluble penetrant component is a polar lipid and the more soluble component is a surfactant or a surfactant-like molecule or else such form of a lipid, preferably a polar lipid which is sufficiently soluble for the purpose of this invention.
20. The use of any one of claims 1 to 19 or the pharmaceutical composition of any one of claims 4 to 19 wherein the average penetrant diameter is between 25 nm and 500 nm, preferably between 30 nm and 250 nm, even more preferably between 35 nm and 200 nm and particularly preferably between 40 nm and 150 nm.
21. The use of any one of claims 1 to 20 or the pharmaceutical composition of any one of claims 4 to 20 wherein the penetrant concentration in the formulation for the use in human or animal nose is 0.001 to 20 weight-% of total dry mass in the formulation, in particular between 0.01 w-% and 15 w-%, more preferably between 0.1 w-% and 12.5 w-% and most preferred between 0.5 w-% and 10 w-%.
22. The use of any one of claims 1 to 21 or the pharmaceutical composition of any one of claims 4 to 21 wherein the supporting medium, e.g. a buffer, is selected to be a biocompatible solution with an osmotic activity similar to that of a monovalent electrolyte with concentration in the range between 1 mM and 500 mM, more preferably between 10 mM and 400 mM, even more preferably between 50 mM and 300 mM, and most preferably between 100 mM and 200

5 mM or else such solution that affords practically sufficient penetrant stability  
combined with practically sufficient transport rate across the barrier.

10 23. The use of any one of claims 1 to 22 or the pharmaceutical composition of any  
one of claims 4 to 22 wherein the relative drug or agent concentration is  
between 0.001 and 40 weight-% of total penetrant mass, in particular between  
0.01 w-% and 30 w-%, even better between 0.1 w-% and 25 w-% and most  
15 preferably between 0.5 w-% and 15 w-%.

20 24. The use of any one of claims 1 to 23 or the pharmaceutical composition of any  
one of claims 4 to 23 wherein the medium supporting the drugs and carriers is a  
biocompatible buffer with pH value between 4 and 10, more frequently between  
5 and 9 and most often between 6 and 8.

25 25. The use of any one of claims 1 to 24 or the pharmaceutical composition of any  
one of claims 4 to 24 wherein the additives are included in the preparation to  
reduce the system sensitivity to chemical, biological or ambient stress, including  
anti-oxidants, antagonists of undesired enzyme action, cryo-preservants,  
30 microbicides, etc., or else modulators of physically important system properties,  
such as formulation viscosity, etc..

35 26. The use of any one of claims 1 to 25 or the pharmaceutical composition of any  
one of claims 4 to 25 wherein the relative drug or agent dose to be administered  
non-invasively through the nose by means of highly adaptable carriers is chosen  
to be between 0.1x and 500x, more often between 0.5x and 250x, and even  
40 more preferably between 1x and 100x different from the corresponding drug or  
agent dose that would have to be injected to achieve the desired biological  
effects.

45 27. The use of any one of claims 1 to 26 or the pharmaceutical composition of any  
one of claims 4 to 26 wherein the applied penetrant dose is between 0.01 mg  
and 15 mg per nostril, even more often is in the range 0.1 mg and 10 mg per  
50 nostril, and preferably is between 0.5 mg and 5 mg per nostril.

- 5 28. The use of any one of claims 1 to 27 or the pharmaceutical composition of any one of claims 4 to 27 wherein the efficiency of administration and the biological effects of the agent or drug chosen are controlled by using different application volumes.
- 10 29. The use of any one of claims 1 to 28 or the pharmaceutical composition of any one of claims 4 to 28 wherein said formulation is administered using a metered delivery device.
- 15 30. The use of any one of claims 1 to 29 or the pharmaceutical composition of any one of claims 4 to 29 wherein different application volumes are selected to control the efficiency of administration and the biological effects of the chosen agent or drug.
- 20 31. The use of any one of claims 1 to 30 or the pharmaceutical composition of any one of claims 4 to 30 wherein the penetrants in suspension are loaded with the drugs or agents within 24 hours prior to the formulation administration, preferably 360 min, more preferably 60 min and even more preferably 30 min before the resulting formulation administration in the nose.
- 25 32. The use of any one of claims 1 to 31 or the pharmaceutical composition of any one of claims 4 to 31 wherein the delivery device is loaded at the treatment site.
- 30 33. The use of any one of claims 1 to 32 or the pharmaceutical composition of any one of claims 4 to 32 wherein the device is loaded separately with penetrants and the molecules, particularly biological agents, to be associated therewith.
- 35 34. The use of any one of claims 1 to 33 or the pharmaceutical composition of any one of claims 1 to 33 wherein the pharmaceutically active ingredient is for administration to the nervous system.
- 40 35. The use or the pharmaceutical composition of claim 34 wherein the nervous system is the brain.
- 45 50 55

- 5 36. The use of any one of claims 1 to 35 or the pharmaceutical composition according to any one of claims 4 to 35 wherein said pharmaceutical composition is a vaccine.
- 10 37. The vaccine of claim 36 which further comprises a pathogen extract or a compound from a pathogen or a fragment or a derivative thereof.
- 15 38. The vaccine of claim 37 wherein said pathogen extract or compound is selected from hepatitis virus, (human) immunodeficiency virus, herpes viruses, small-pox (chicken-pox), influenza, measles, mumps or polio viruses, cytomegalovirus, rhinovirus, etc., or fungi prospering inside host cells, a parasite including animal parasites, such as protozoa and helminths, and ectoparasites, such as ticks and mites, or *Brucella* species, including the causative agent for cholera, *Haemophilus* species, as well as pathogens triggering paratyphoid, plague, rabies, tetanus or rubella diseases.
- 20 39. The vaccine of any one of claims 36 to 38 which further comprises an adjuvant.
- 25 40. The vaccine of claim 38 or 39 wherein said adjuvant is lipopolysaccharide, such as lipid A or a derivative or modification thereof, such as monophosphoryl lipid A, or its analogue, such as a fatty derivative of saccharose, cord-factor (trehalose-dimycolate), muramyl dipeptide, or another (poly)saccharide or (poly)peptide identical to or resembling an immunologically active part of a membrane of a microorganism; an extract of a microorganism, including bacterial exo- and endotoxins, preferably cholera toxin or the heat labile toxin of *E. coli*, an A-chain derivative, a component with an ADP-ribosylating activity, a peptidoglycane, a clostridial toxin, an LT halotoxin, purified protein derivative of *M. tuberculosis*, LT-R192G, Fibronectin-binding protein I of *Streptococcus pyrogenes*, or outer membrane protein of group B *Neisseria meningitidis* (GBOMP), bacterial or viral nucleic acids, such as oligonucleotides comprising unmethylated CpG dinucleotides.
- 30 41. The vaccine of any one of claims 36 to 40 comprising a blend of MPL and IL-12 or GM-CSF and IL-4.
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- 5 42. The vaccine of any one of claims 36 to 41 wherein the relative immunogen/antigen dose to be administered non-invasively through the nose by means of highly adaptable carriers is chosen to be between 0.01x and 100x, more often between 0.05x and 75x, and even more preferably between 0.1x and 10 50x different from the corresponding immunogen/antigen dose that would have to be injected to achieve the desired biological effect.
- 15 43. The vaccine according to any one of claims 39 to 42 wherein the concentration of the transnasally administered adjuvant is between 10x lower and up to 1000x higher than that used with the corresponding subcutaneously injected formulations employing similar antigen, the transnasally administered 20 immunoadjuvant concentration more often differing from the injected immunoadjuvant concentration by the factor between 0.5 and 100, or better, by the factor between 1 and 50, and best between 2 and 25.
- 25 44. A container comprising the pharmaceutical composition according to any one of claims 4 to 43.
- 30 45. A package comprising at least one container comprising the pharmaceutical composition of any one of claims 4 to 43.
- 35 46. A method for generating a protective immuno response on a mammal by vaccinating said mammal with a vaccine according to any one of claims 36 to 43.
- 40 47. The method according to claim 46 wherein different administration volumes are selected to control the applied immunogen dose and the outcome of vaccination.
- 45 48. The method according to claim 46 or 47, wherein a suspension of antigen-free penetrants is loaded with the antigen to be associated therewith during the day prior to an administration, preferably 360 min, more preferably 60 min and even 50 more preferably 30 min before administering the resulting formulation in the nose.
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- 5 49. The method of any one of claims 46 to 48 characterised in that at least one dose of vaccine is administered.
- 10 50. The method according to claim 49 wherein said vaccine is administered as a booster vaccination.
- 15 51. The method according to any one of claims 46 to 50, wherein the vaccine is applied between 2 and 10, preferably between 2 and 7, even more preferably up to 5 and most preferably up to 3 times, when a non-allergenic antigen is used, or such a number of times, in the case of allergens, as is required either to achieve the desired immuno-tolerance, determined according to a suitable assessment method, or else to deem the effort as having failed.
- 20 52. The method according to any one of claims 48 to 51, wherein the time interval between the subsequent vaccinations is chosen to be between 2 weeks and 5 years, often between 1 month and up to 3 years, more frequently between 2 months and 1.5 years.
- 25 53. The method according to any one of claims 46 to 52, wherein the flux of penetrants that carry an immunogen through the various pores in a well-defined barrier is determined as a function of a suitable driving force or a pressure acting across the barrier and the data are then conveniently described by a characteristic curve which, in turn, is employed to optimise the formulation or application further.
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Intranasal application of Transfersulin  
IDDM test person, -0.8 IU/kg

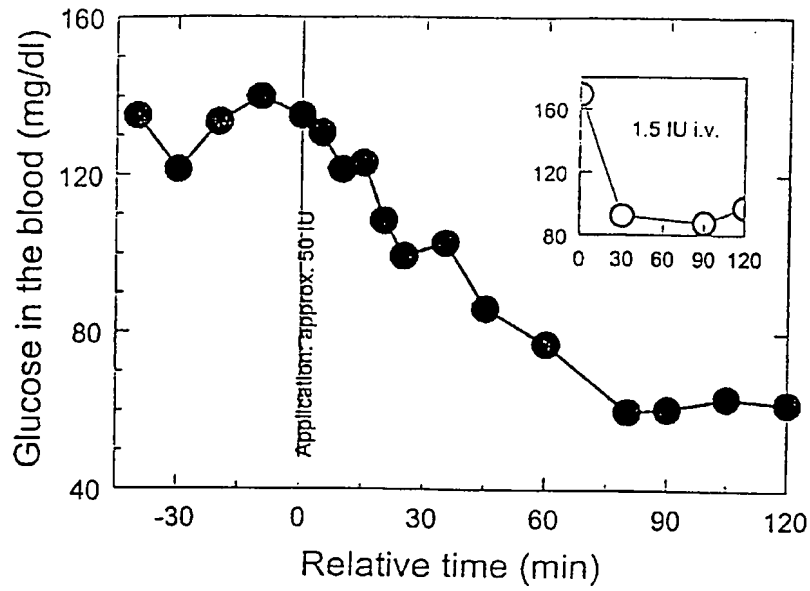


Fig. 1

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Intranasal application of Transfersulin  
on a normoglycaemic test person, ~ 0.15 IU/kg (2x)

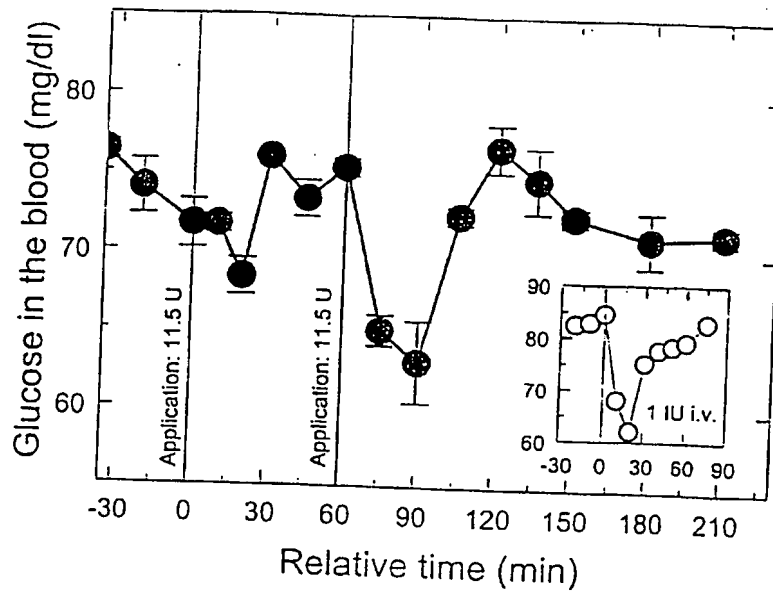


Fig. 2

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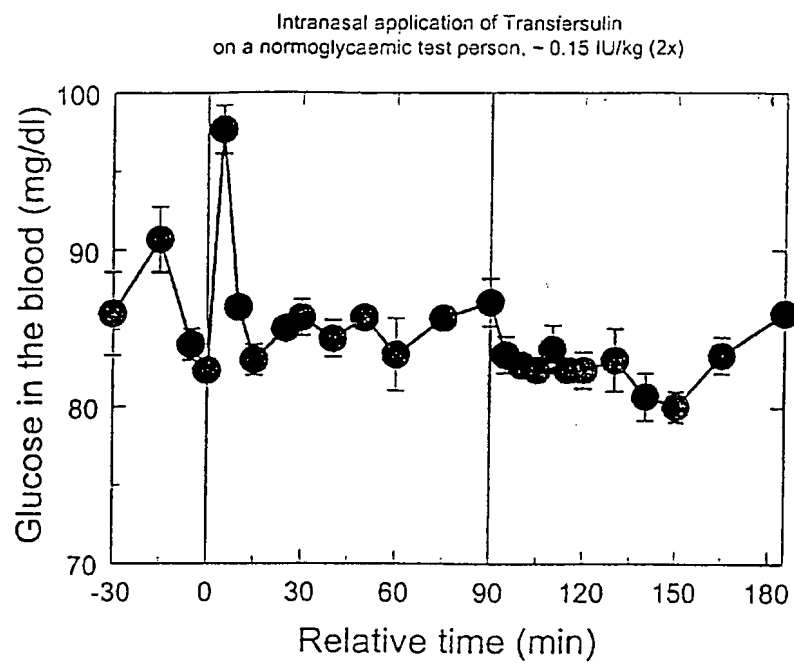


Fig. 3a

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Intranasal application of Transfersulin  
In IDDM test person, -0.4 IU/kg

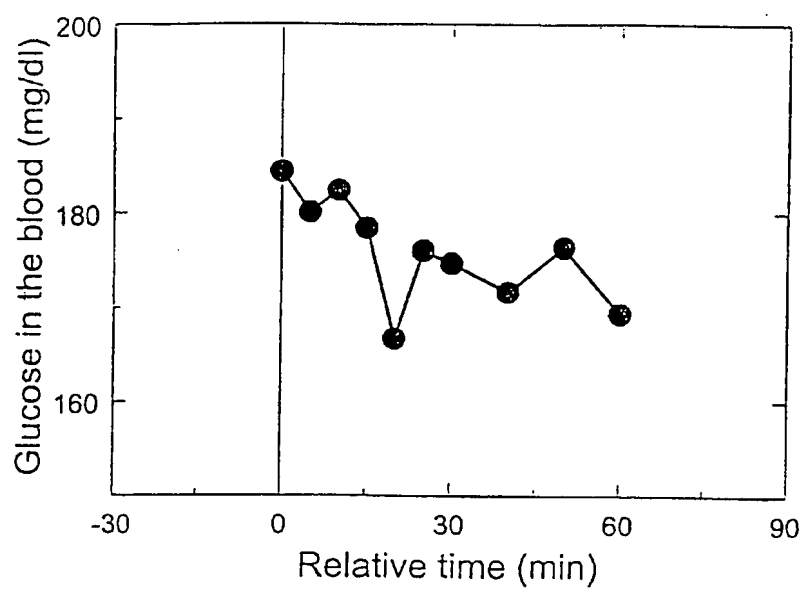


Fig. 3b

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Effects of nasally administered  
cytokines on specific immune response,  
1st boost + 7 d

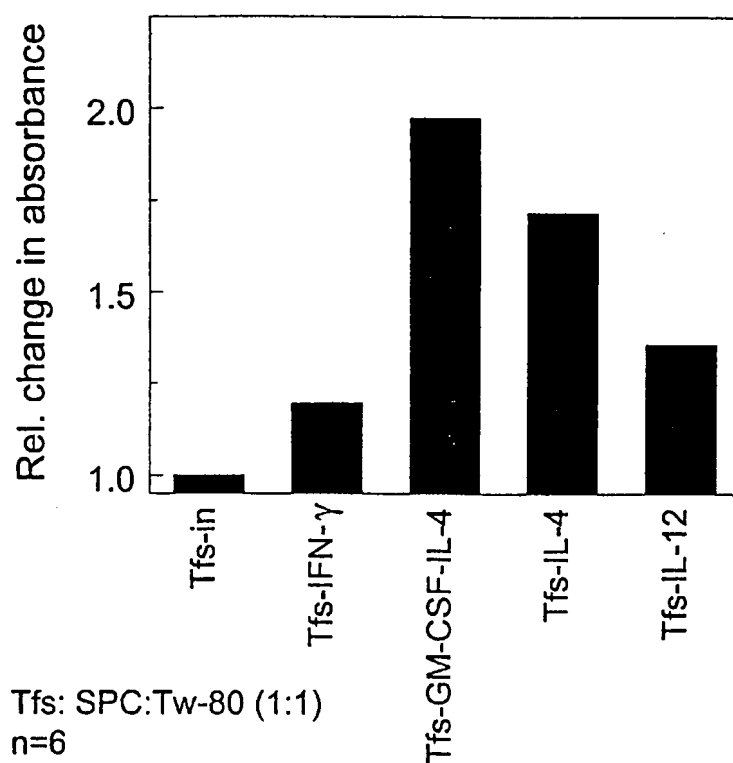


Fig. 4

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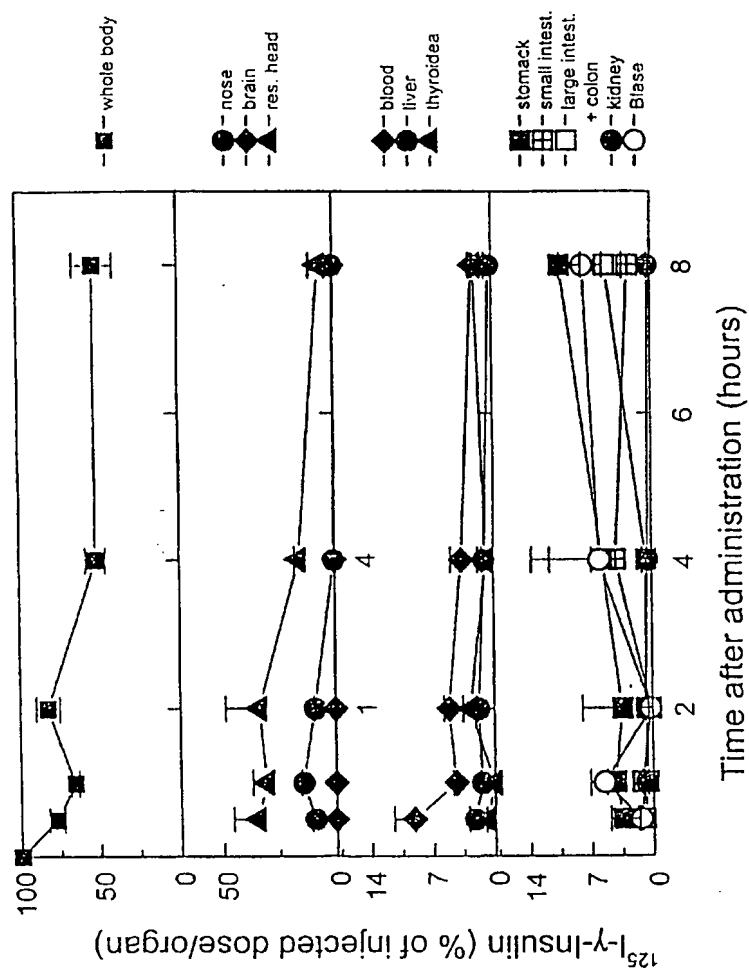


Fig. 5

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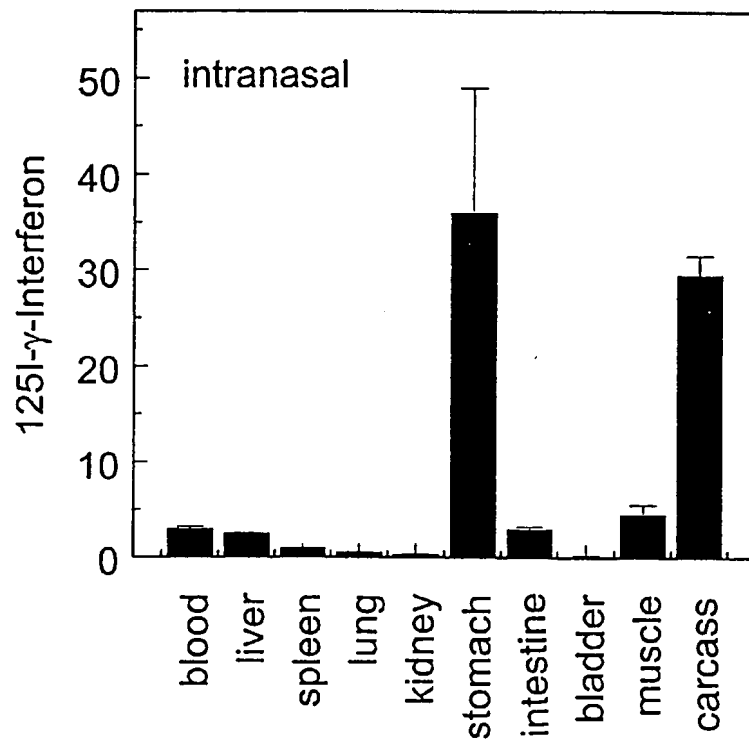


Fig. 6



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Intranasal administration: Tetanus toxoid (TT, impure)

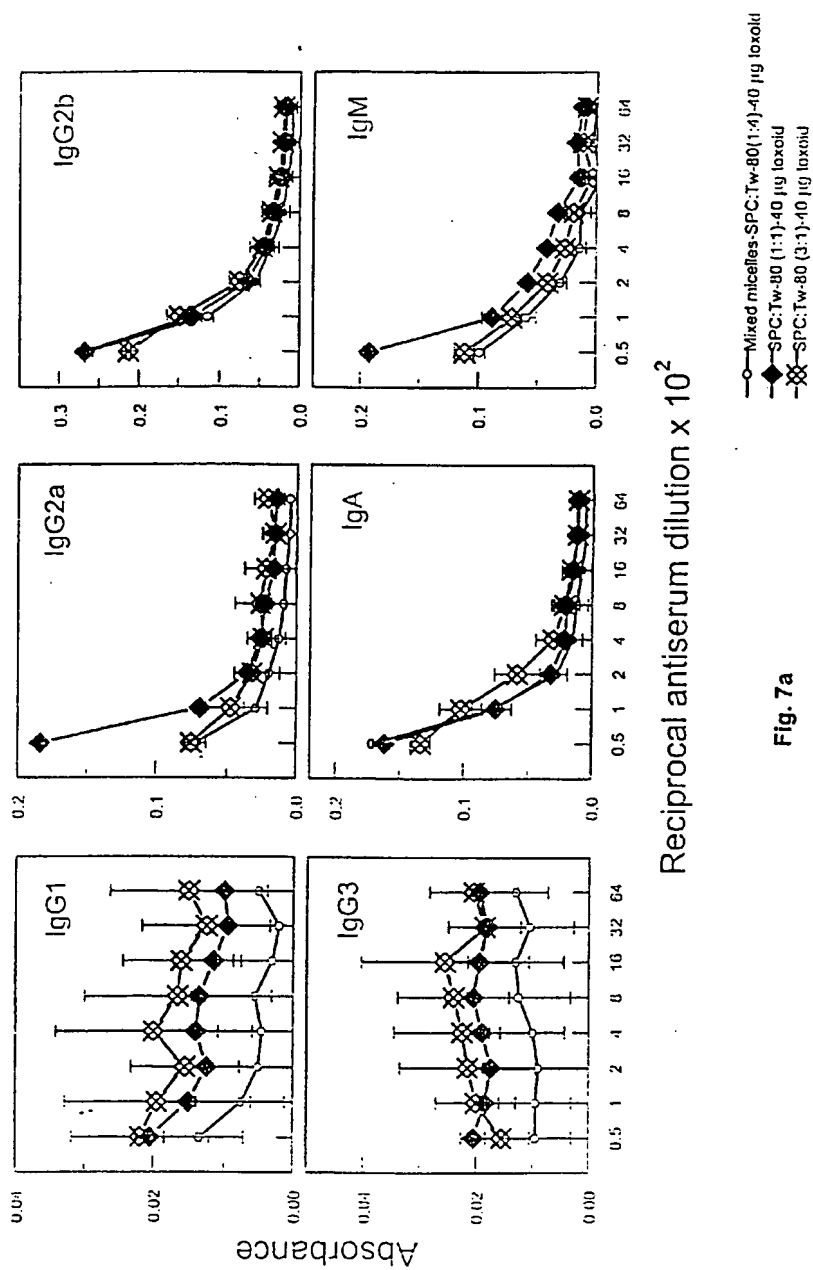


Fig. 7a

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Intranasal immunisation: Tetanus toxoid (TT impure)

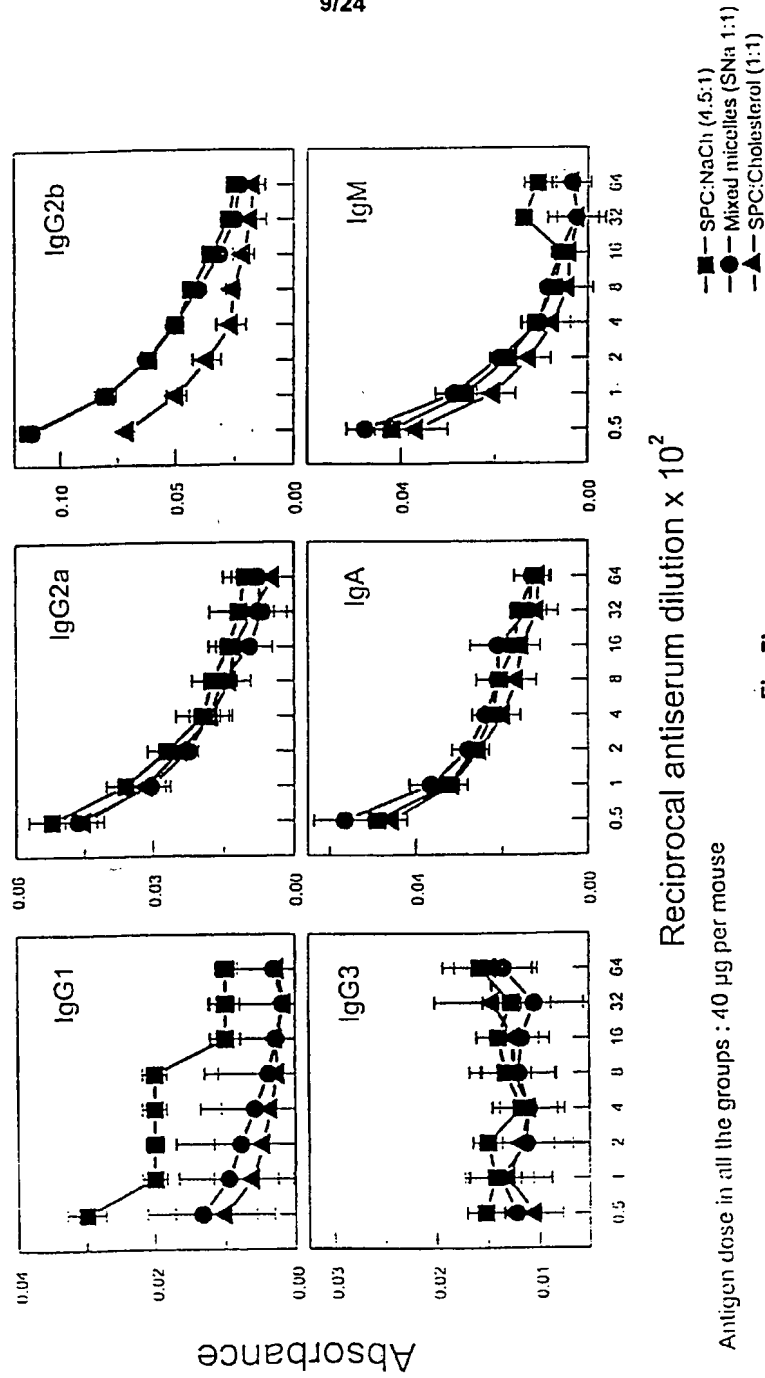


Fig. 7b

Antigen dose in all the groups : 40 µg per mouse

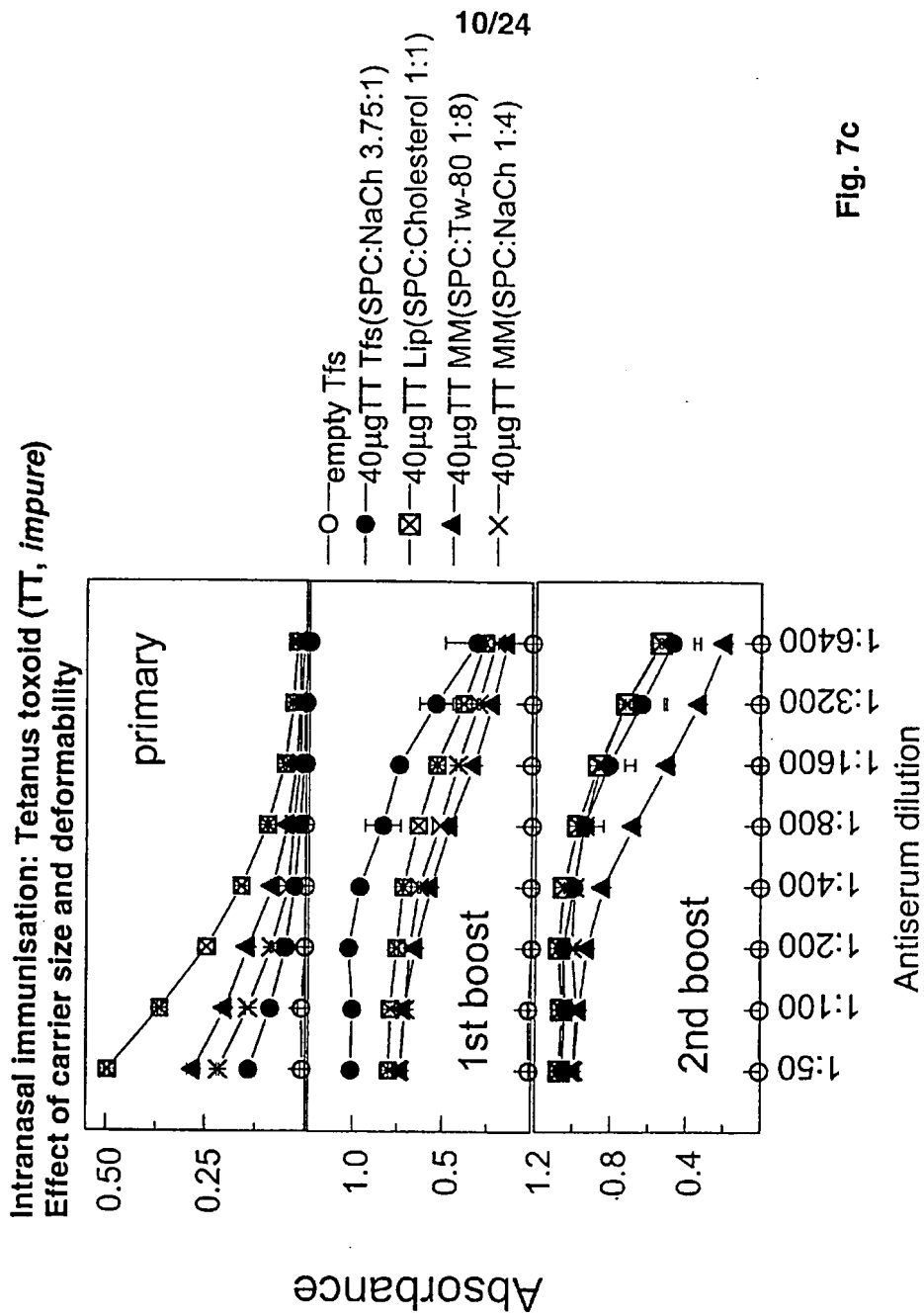


Fig. 7c

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Intranasal Immunization: Tetanus toxoid, (TT, purified)  
dose effect

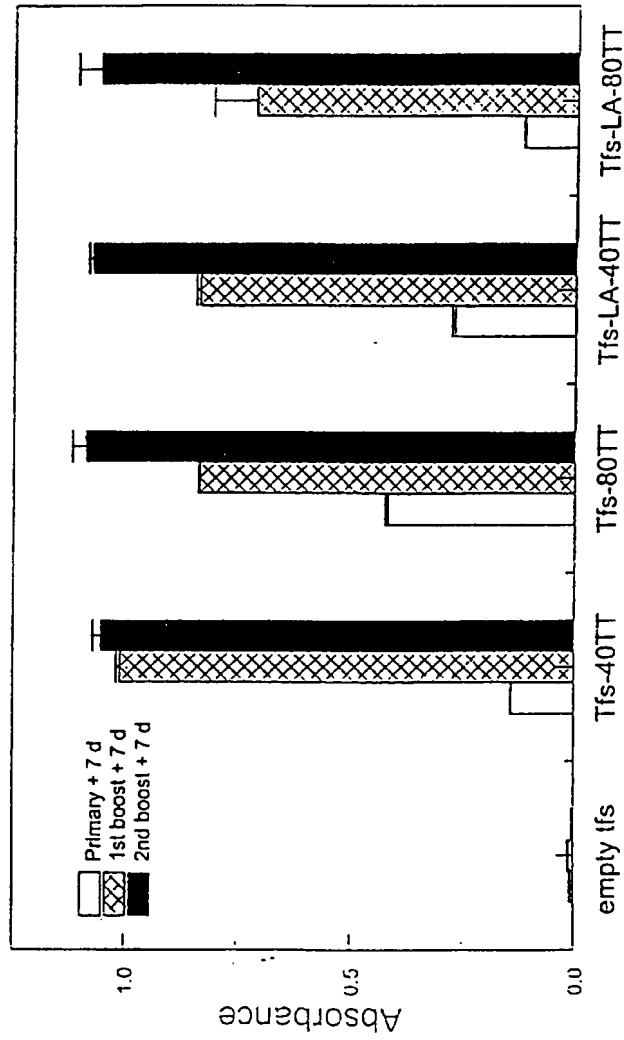


Fig. 8a

Intranasal immunisation: Tetanus toxoid (TT, purified), Effect of antigen dose

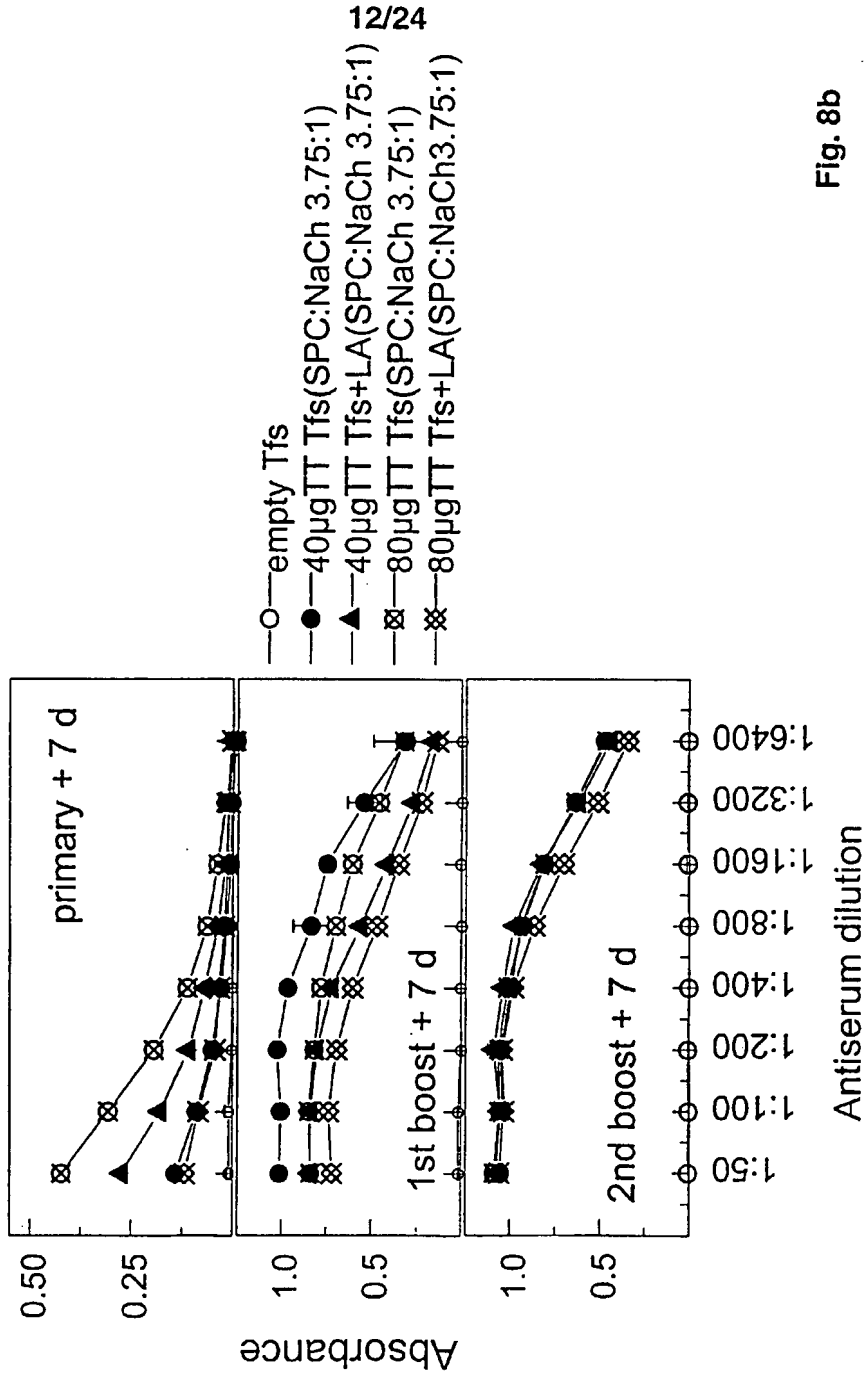


Fig. 8b

Intranasal Immunization: Tetanus toxoid (TT, purified), dose effect

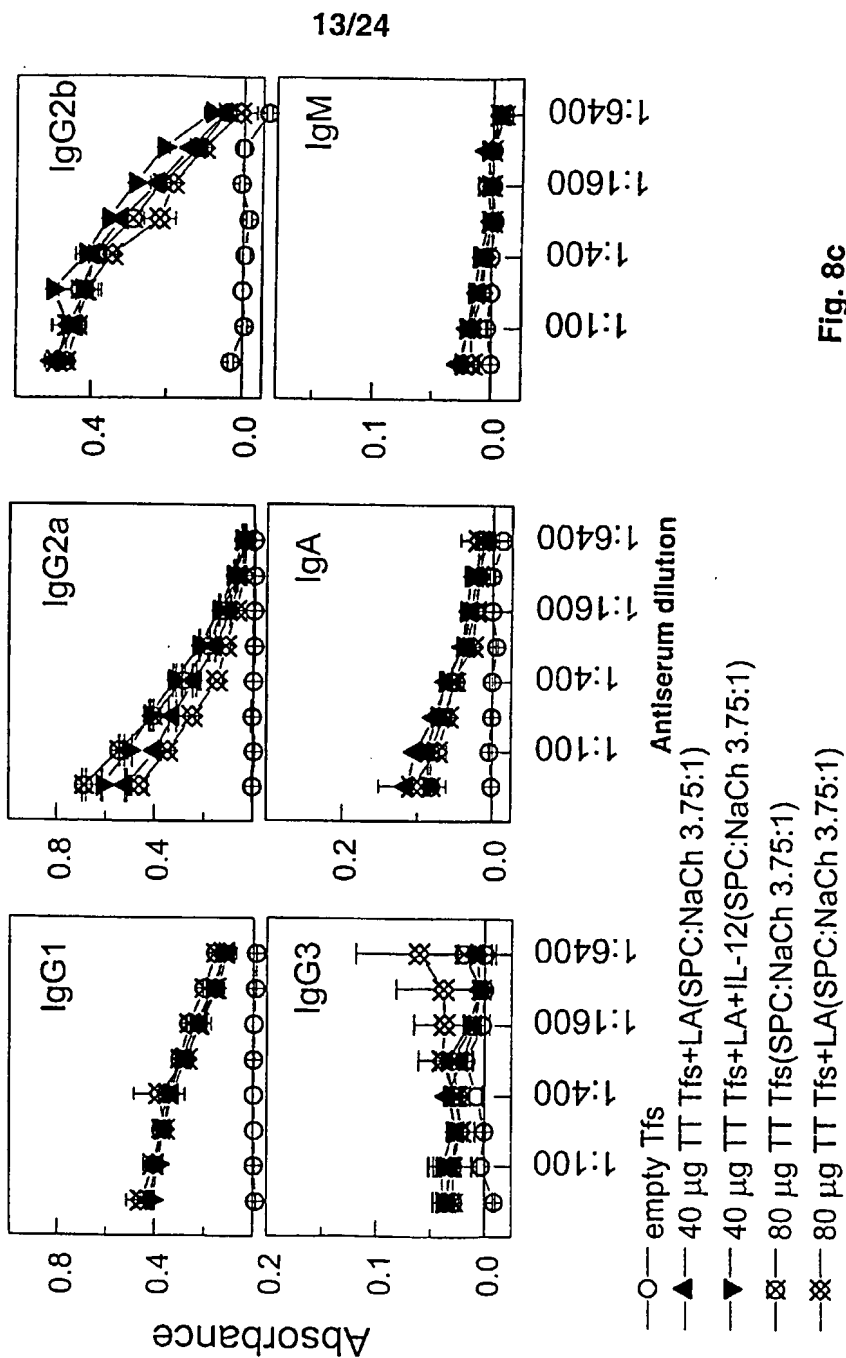


Fig. 8c

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Noninvasive immunisation: Tetanus toxoid (TT, purified)  
effect of application route

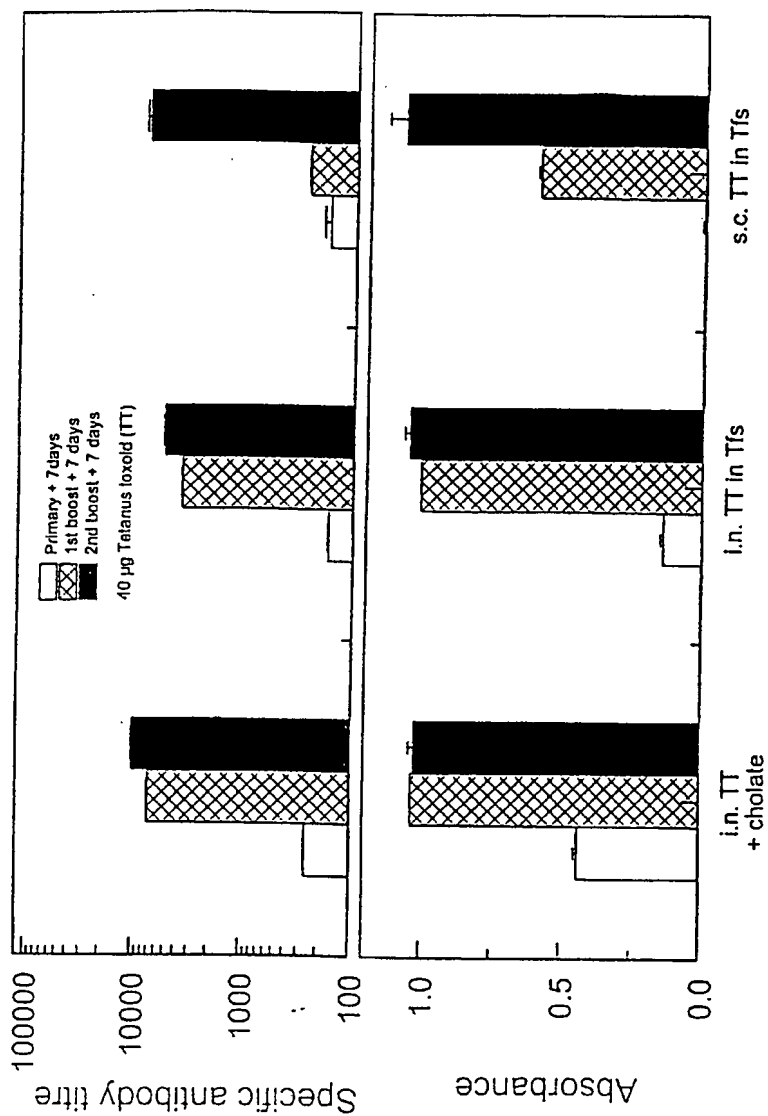


Fig. 9a

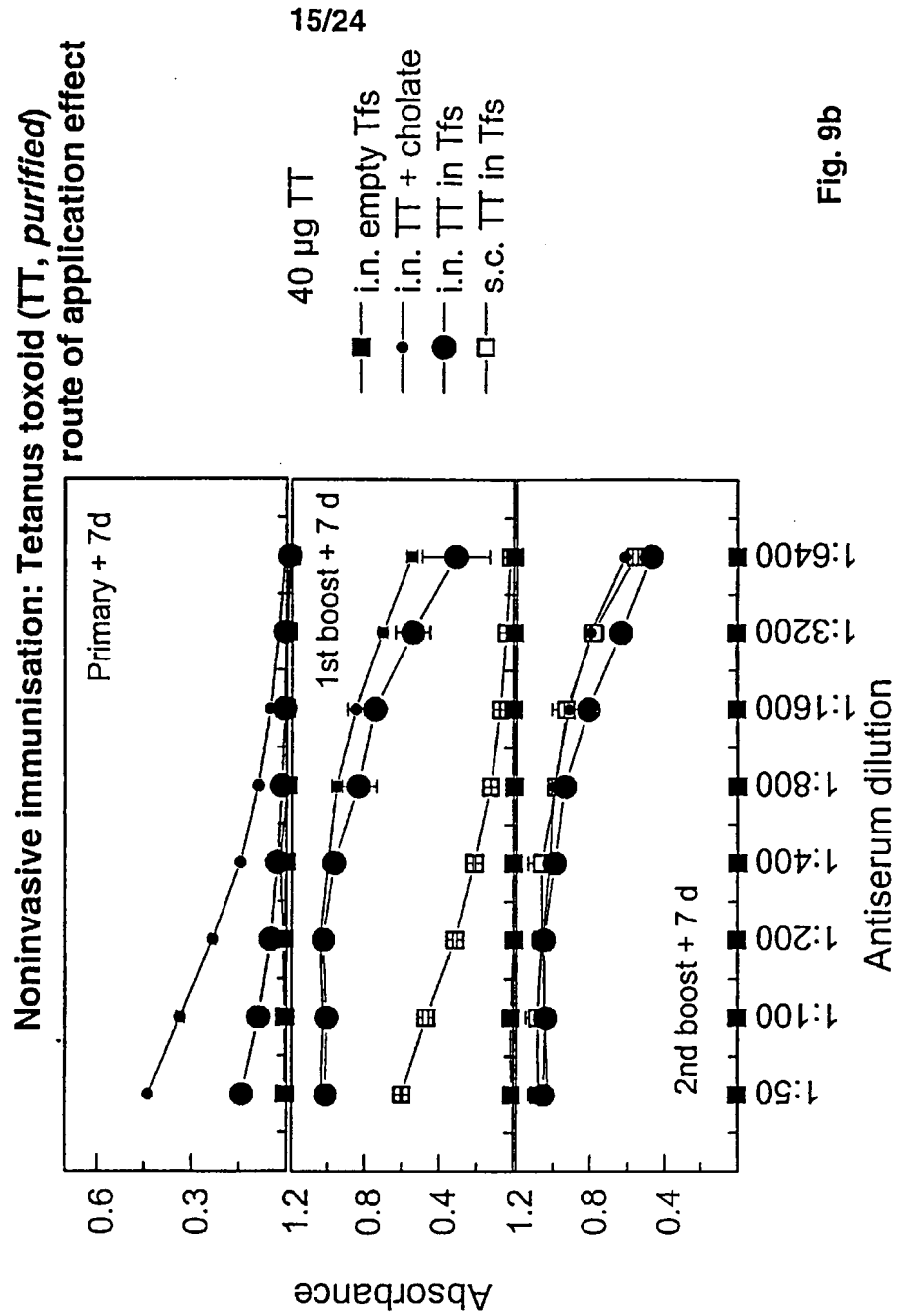


Fig. 9b



## Antigen-specific Isotyping: Intranasal / Subcutaneous

40 µg Tetanus Toxoid (TT), ultrafiltered

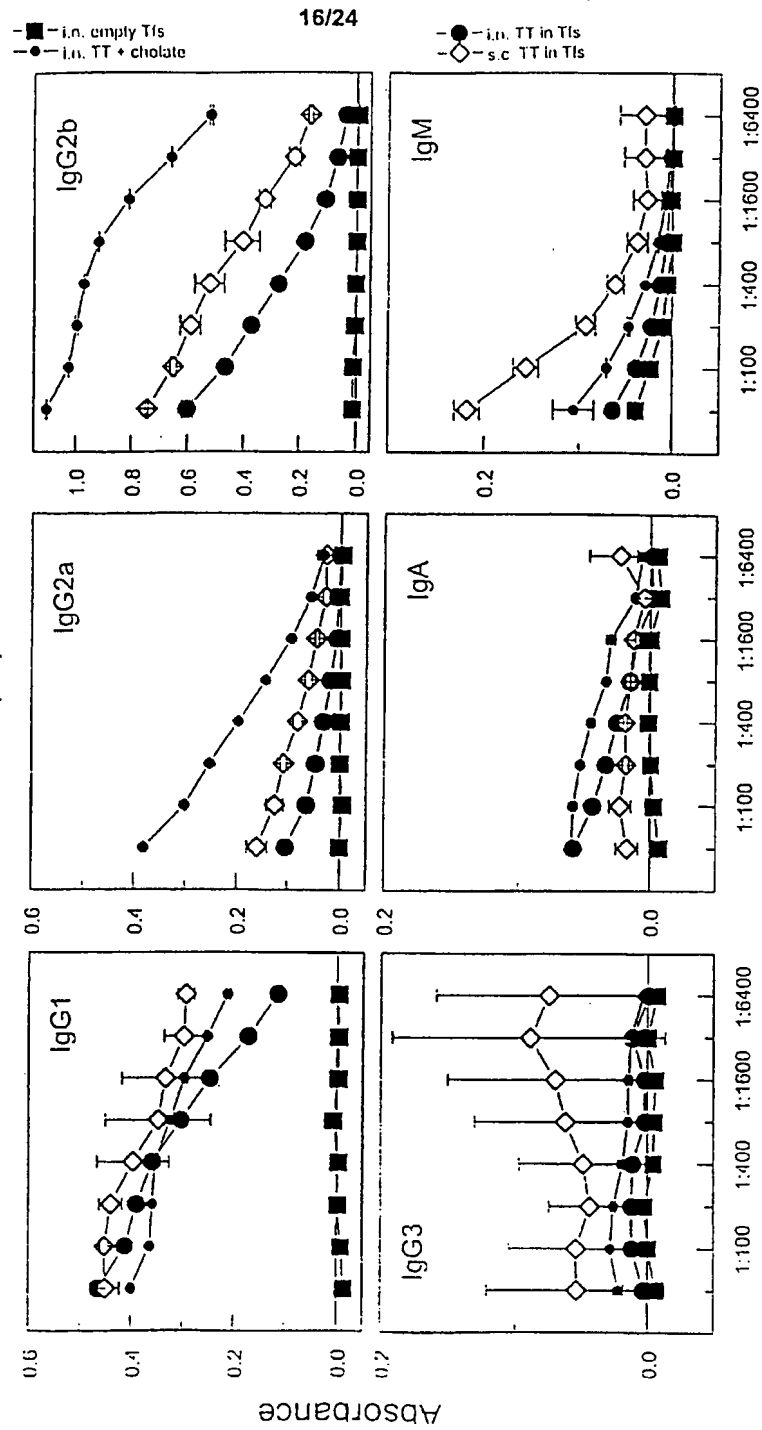


Fig. 9c

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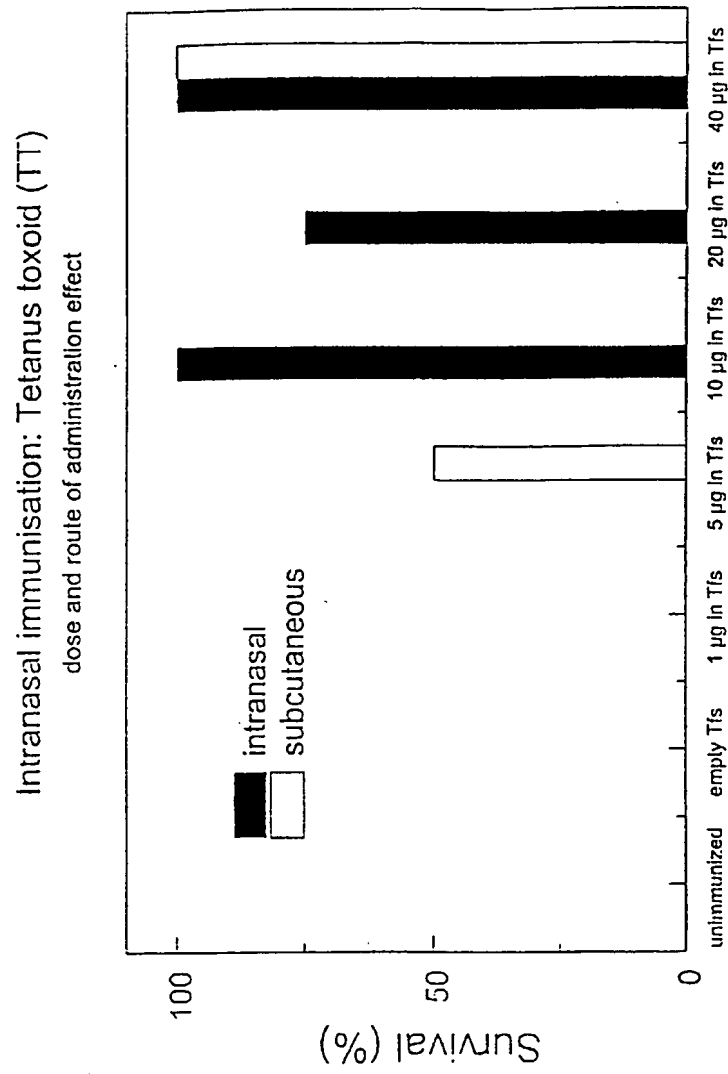


Fig. 10a

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Intranasal immunisation: Tetanus toxoid (TT, purified) dose effect

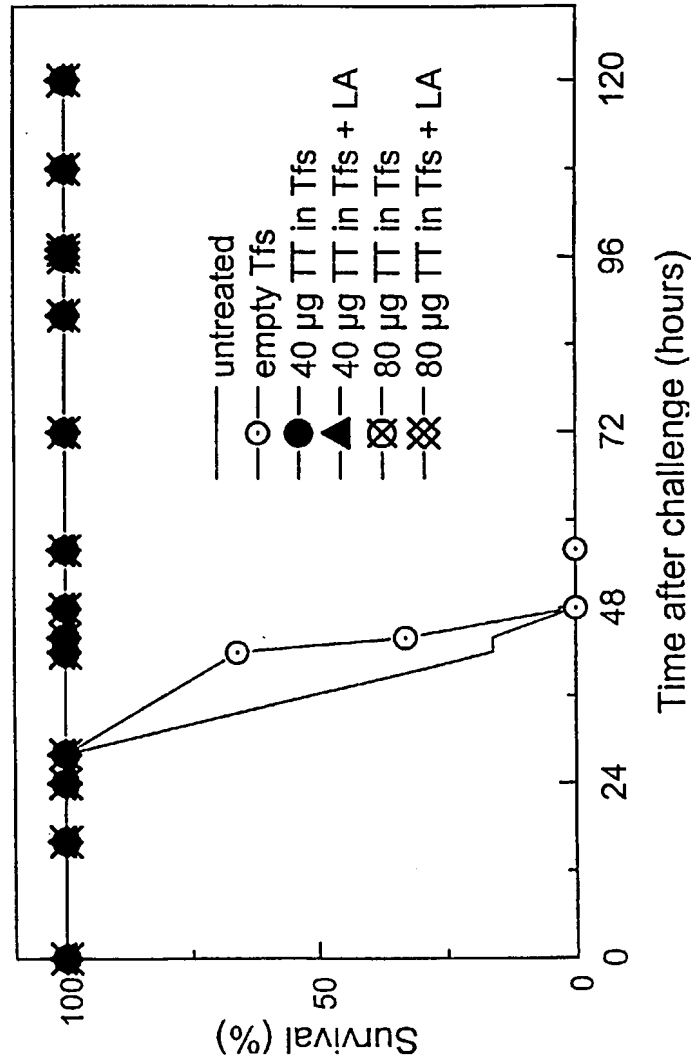


Fig. 10b

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Intranasal immunisation: Tetanus toxoid (TT, *impure*)  
effect of immunomodulators

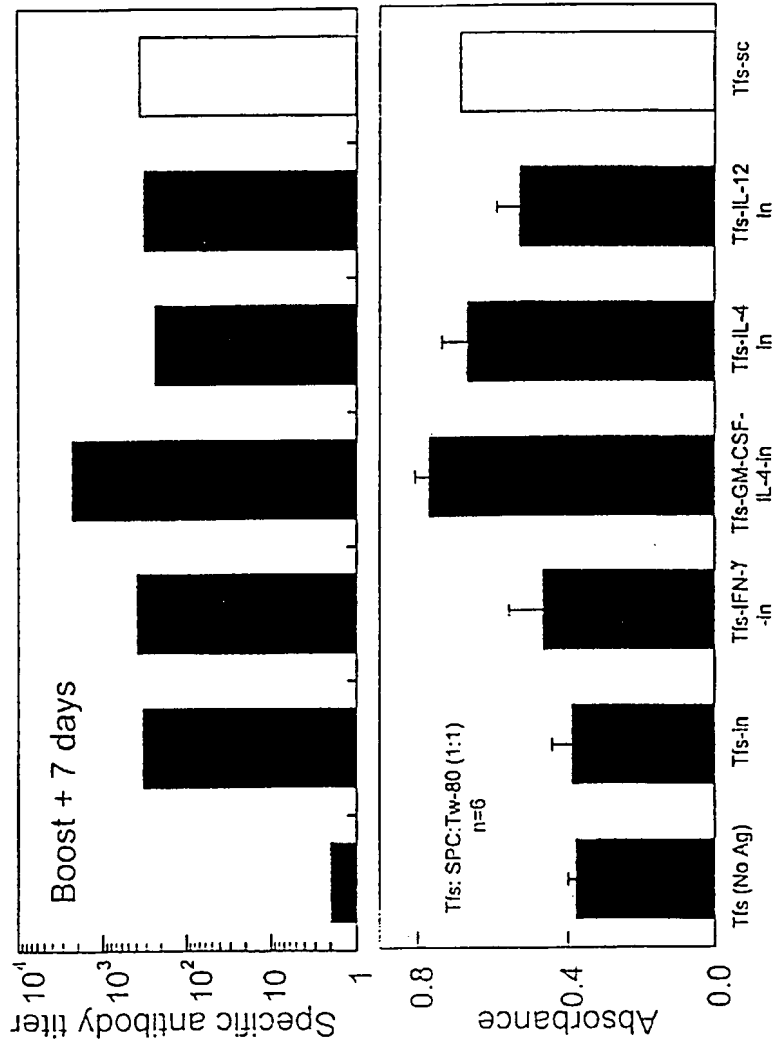


Fig. 11a

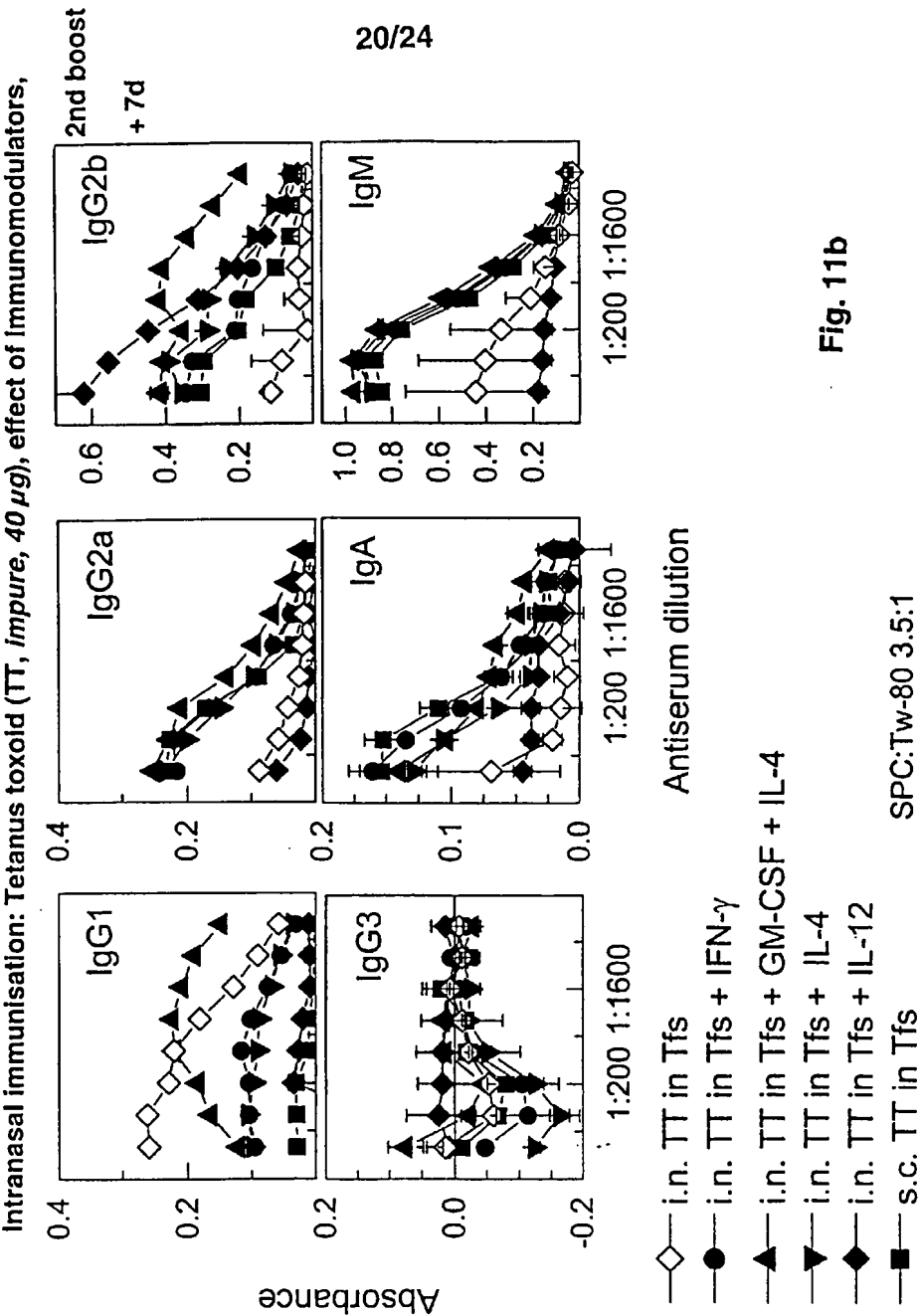


Fig. 11b

Intranasal immunisation: Tetanus toxoid (TT, purified)  
effect of immunopotentiators

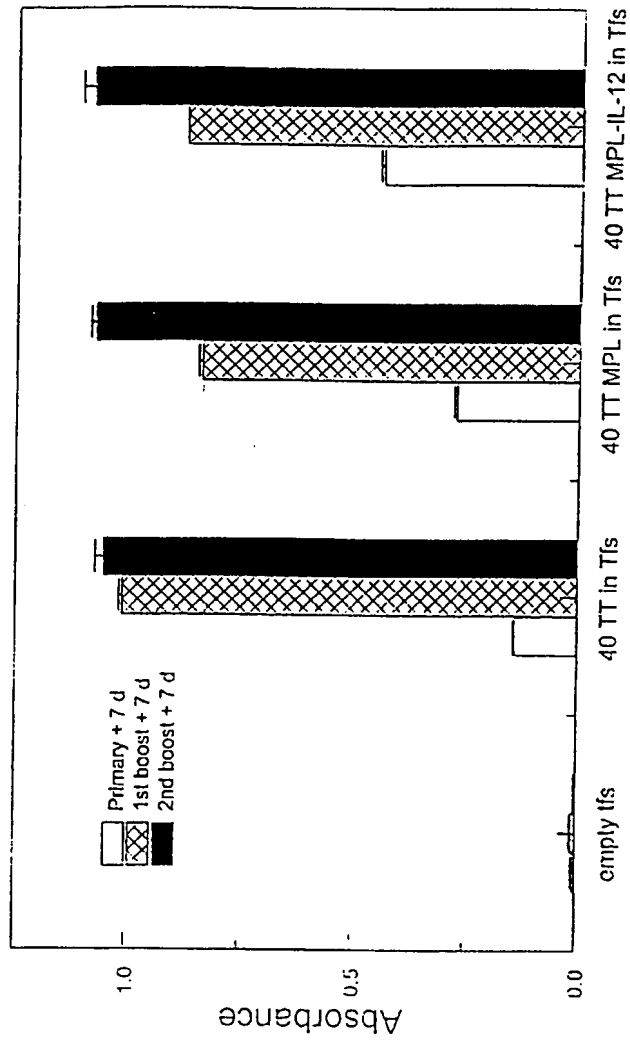


Fig. 12

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**Potential of nasal immune response to tetanus**  
**toxoid (TT) by bacterial adjuvant, cholera toxin (CT)**

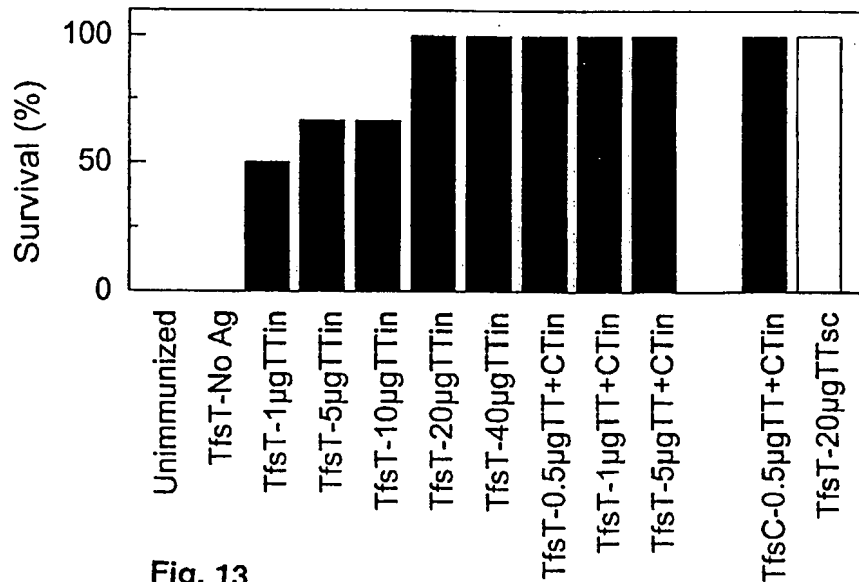
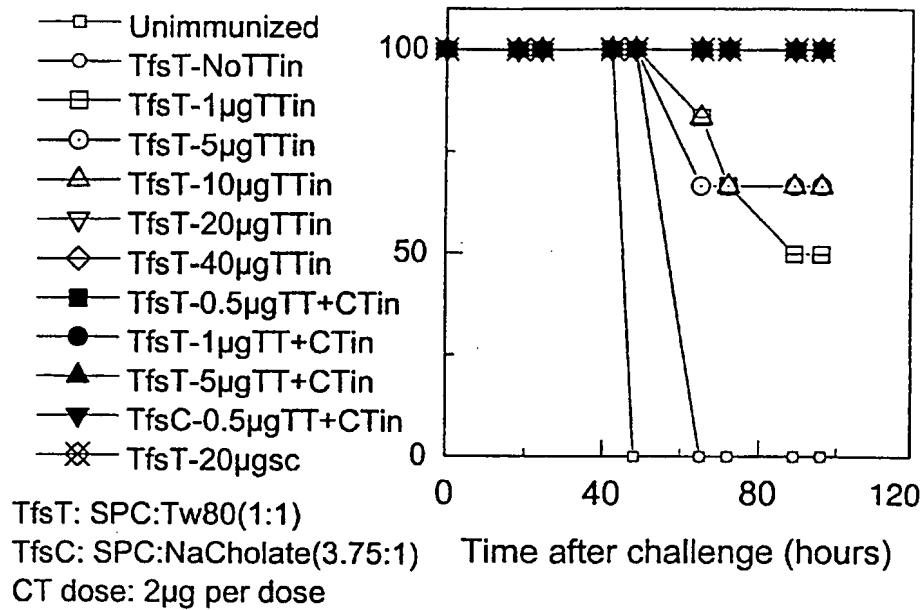
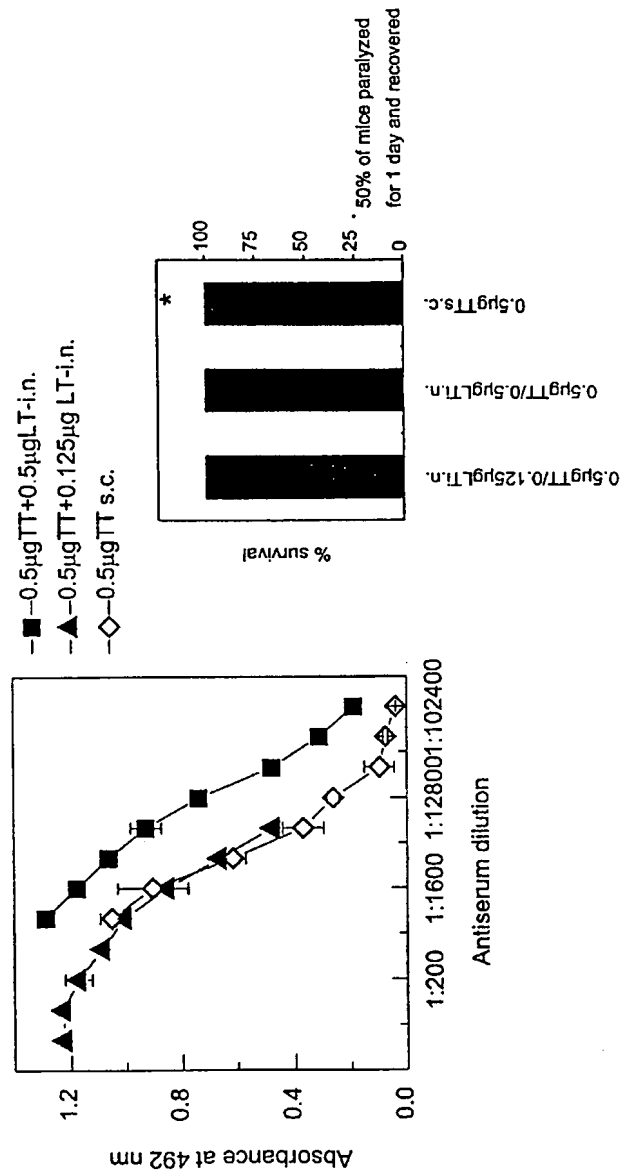


Fig. 13





**Bi-Valent Vaccines: Anti-Tetanus and -Cholera response**  
to the antigens administration in Transfersomes in the nose

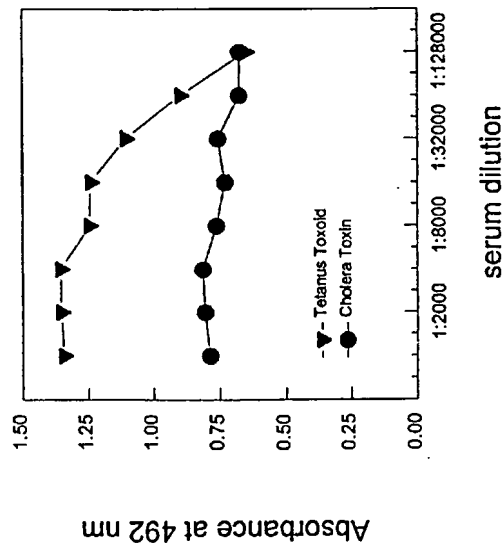


Fig. 15

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/EP 00/00598

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K9/127 A61K38/19 A61K39/39 A61K38/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 475 160 A (CEVC GREGOR) 18 March 1992 (1992-03-18) cited in the application page 1, line 1-6 examples claims  —  -/-	4,5

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

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- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

15 June 2000

Date of mailing of the international search report

28/06/2000

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Muller, S

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/00598

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 90 09385 A (LIPOSOME CO INC) 23 August 1990 (1990-08-23) page 3, line 17-34 page 9, line 6-17 claims	1
A	WO 92 04009 A (UNIV LONDON PHARMACY) 19 March 1992 (1992-03-19) page 1, line 3-7 page 3, line 21-32 page 6-7; example 1 page 14; example 2 claims	1-53
A	CEVC 6: "Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery" CRITICAL REVIEWS IN THERAPEUTIC DRUG CARRIER SYSTEMS, vol. 13, no. 3-4, 1996, pages 257-388, XP002107366 page 316 -page 321	1

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Information on patent family members

Inter. Appl. No.

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